PRE-CLINICAL DETECTION AND TREATMENT TARGETING IN COLORECTAL HEPATIC METASTASES

A thesis submitted to the University of Glasgow

for the degree of M.D. in

the Faculty of Medicine

By

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ACKNOWLEDGEMENTS

I wish to thank all my colleagues in Glasgow Royal Infirmary, University Department of Surgery, whose friendship I will never forget. More specifically,I will be eternally grateful to Professor Timothy Cooke and Wilson Angerson for their supervision,enthusiasm, guidance and endeavour.

I also appreciate the contributions of David Hemingway, whose established models I inherited, and particularly John H.Anderson, without whose help the complexities of the cross perfusion experiment would have overcome my determination.

I am indebted to Gordon Murray,Carol and Audrey of the University Department of Statistics for their guidance and assistance in analysing of the results.

I am grateful to Robert,who always managed to find what I needed;to Peter whose "Mac" was invaluable;to Bruce,David,Colin, and the boys whose healthy cynicism maintained my perspective;and to the girls in my "coffee room" for providing gossip and solace when required.

Last,but not least,my family - my father(Kevin),Pat and sister(Dawn),and particularly my wife(Lynne),who have all been patient and tireless in their encouragement. However it is to my mother,Anita,to whose memory this thesis is dedicated.

ABSTRACT

The prognosis for patients with colorectal metastases at the time of presentation is poor. Recently published reports have suggested that moderate improvements in survival are possible in patients with colorectal carcinoma, using 5-FU based chemo-therapeutic regimes given either as adjuvant therapy or in the presence of established metastases. The benefit is stage specific,less than 50% of patients being suitable candidates,the treatment of all patients therefore resulting in unnecessary morbidity,increasing the importance of accurate peri-operative staging.

Most currently available imaging techniques are "density dependent", detecting a difference in the density of tumour deposits,relative to the surrounding liver,but these rarely detect lesions less than 1 cm in diameter,and will consequently miss patients with micrometastatic disease. Dynamic hepatic scintigraphy is an indirect method of measuring liver blood flow and can distinguish the relative contribution of the portal venous and hepatic arterial components of liver blood flow. The ratio of the hepatic arterial contribution to total liver blood flow is termed the "Hepatic Perfusion Index"(HPI).

The HPI has been shown to be raised in the presence of overt metastases and also in a proportion of those patients who are apparently clear at the time of presentation but who subsequently develop hepatic metastases. The reproducibility of the standard hepatic scintigraphy technique(80 MBq administered activity) has been questioned and the technique has not become established in clinical practice.

A high administered activity(400 MBq) modification of dynamic scintigraphy technique has been assessed showing improved correlation following repeated analysis of studies by a single observer,and also on separate analysis by two observers. A prospective imaging study comparing five imaging modalities (U/S,CT,Static scintigraphy,dynamic scintigraphy and intraoperative ultrasound) with intraoperative palpation confirmed that the HPI was as accurate as contrast enhanced CT in the detection of colorectal metastases. However,the specificity was poor.

The change in the HPI has been shown in a rat tumour model,to be as a result of a reduction in splanchnic vascular inflow due to an increase in the splanchnic vascular resistance. A similar effect has been shown in patients. A cross perfusion model was developed to determine if the increase in the splanchnic vascular resistance was due to a transmissible humeral agent. The perfusion pressure was similar in controls and tumour bearing animals,but the flow produced was reduced during periods of perfusion with tumour bearing blood due to a significant increase in the vascular resistance,confirming the presence of a circulating agent.

Using a reference microsphere technique,the splanchnic vascular flow was shown to reduce in animals with both intrahepatic and extrahepatic(flank) tumour.The hepatic arterial flow was reduced in animals with extrahepatic tumour but not intrahepatic tumour,suggesting the change in the HPI in the presence of intrahepatic tumour was due to local(hepatic arterial) protection of flow despite splanchnic vascular vasoconstriction.

The efficacy of present chemotherapeutic regimes is limited by toxicity. By localising the drug in the target tissue,dose escalation is possible due to reduced

systemic exposure. First order targeting(to the organ)usually requires a major surgical procedure to insert a gastroduodenal artery catheter. Organ targeting may in theory be achieved biochemically taking advantage of the normal biochemical pathways of the body avoiding the morbidity of surgery. Three potential targeting vehicles of this type were assessed in a rat model of hepatic metastases.

In the first study,^{14}C labelled 5-FU bound to a manose/gaurin conjugate with secondary galactose residues was assessed as a targeting system. There was little advantage over free drug in achieving hepatic targeting,and the venous pharmacokinetic profile was suggestive of rapid breakdown of the 5-FU/gaurin complex.

A similar series of experiments, using a ^{125}I labelled hydroxymethyacrylamide (HPMA) polymer, again with secondary galactose residues, confirmed organ specific targeting could be achieved in this way, with up to 60% of the polymer localising in liver within 30 minutes. Levels within intrahepatic tumour were disappointing. Regional delivery of the polymer had little effect on subsequent distribution,and although delivery via the hepatic artery increased the concentration within tumour tissue, levels were still less than 10% of surrounding liver parenchyma.

Porphyrin metabolism results in localisation within the reticulo-endothelial system and within tumour tissue. Again over 50% of a ^{14}C-labeled haematoporphyrin derivative could be detected within the liver. With HpD tumour levels were better than with HPMA, being nearly half of that in surrounding liver. Further refinement is required to achieve positive tumour to liver ratios.

Regional administration of drugs via a surgically implanted catheter is designed to improve drug delivery to tumour. This may be further refined by the

coadministration of either degradable microemboli or arterial vasocontrictors,which divert drug from normal liver,and/or delay its washout. The benefit from vasocontrictors is known to be temporary, the effect being minimal 90 minutes following administration. The effect of combining these two approaches was studied in an animal model of colorectal metastases. The administration of the angiotensin II prior to the degradable starch microspheres proved the most effective method of targeting a radiolabelled marker substance. The combination significantly increased both the absolute retention of marker in tumour and the tumour : liver ratio over animals treated with DSM alone.

DECLARATION

I declare that all experiments,investigations,unless stated in the text,and analysis of the data have been performed personally. In addition,no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification to this or any other University,

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CHAPTER 1

INTRODUCTION

INCIDENCE OF COLORECTAL CARCINOMA

Colorectal carcinoma is the third most common carcinoma behind lung and skin cancer in men and breast and lung carcinoma in females. Each year in Britain there will be 22,000 new cases,900 in the West of Scotland (W.S.C.S.U.,1990),only 9,000 of which will be cured by surgical resection.

The prognosis in patients is largely determined by the extent of spread at the time of diagnosis. Tumours confined to the bowel wall have a five year survival in excess of 90%(Grem,1991),but these unfortunately account for less than 10% of cases(Umpleby et al,1984;Gill and Morris,1978),the majority having spread at least through the bowel wall,and approximately 20% having palpable metastases at laparotomy,who have a median survival of less than 6 months(Wood et al,1976;Bengmark and Hafstrom,1969).

Local recurrence in the original tumour bed represents a major component of failure, however attention to the detail of the surgical procedure(McGlone et al,1982),or the addition of adjuvant radiation may produce improvements in local control. Patients with disease limited to the original tumour bed comprise less than a quarter of all patients dying of colorectal carcinoma,the remainder having disseminated disease. Two-thirds of these patients will have hepatic metastases.

In the last two decades the percentage of patients known to have metastases at presentation has increased from 18-19% (Oxley and Ellis,1969;Bengmark and Hafstrom,1969) to around 30% (Finlay and McArdle,1986;Machi et al,1987) due to improved imaging techniques. The proportion of patients that are undergoing

"potentially curative" surgery in the presence of undetected micrometastases is therefore reducing. Despite this,more than 20% will subsequently develop recurrent disease,one third with local recurrence,one third with hepatic or distant metastases,the remainder with a combination of both(Rich et al,1983).

The development of hepatic metastases depends on the dissemination of malignant cells from the primary tumour into the mesenteric tributaries and subsequently into the portal vein,before embedding in the hepatic bed. In those patients in whom surgery fails, it is assumed that micrometastases exist that are responsible for metachronous metastases. Whether these metastatic deposits are already present at the time of surgery,but below the threshold of detection,or whether dissemination occurs at the time of surgery is unresolved. Radical surgery or employing early isolation,"no touch" technique has more effect on local recurrence than distant dissemination(McGlone et al,1982;Turnbull,1980). Either way, these microdeposits have a high growth fraction and should be therefore be at their most sensitive to cytotoxic agents.

Table 1. Relative frequency of common cancers in the West of Scotland:

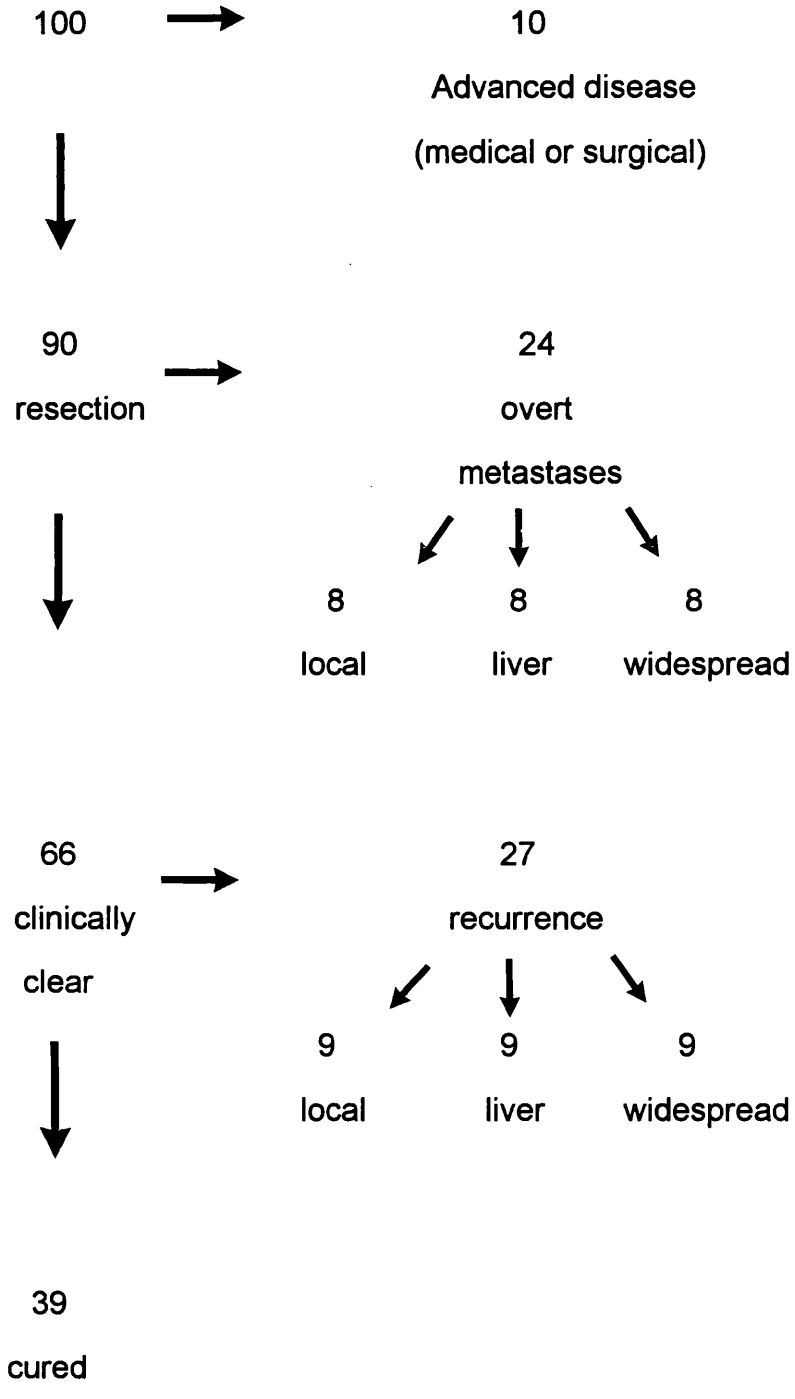
Males		Females	
Lung	140	Breast	93
Skin(Ex.melanoma)	53	Lung	64
Colon,Rectum	48	Colon,Rectum	54
Prostate	43	Skin(Ex.melanoma)	44
Bladder	34	Stomach	19
Stomach	27	Ovary	19
Oesophagus	15	Cervix	17
Lymphoma	13	Bladder	14
Pancreas	12	Pancreas	12
Kidney	11	Oesophagus	10
Leukaemia	10	Endometrium	10
Oral tumours	9	Lymphoma	10
Larynx	8	Leukaemia	9
Brain	6	Melanoma	9
Melanoma	6	Kidney	6

Rates per 100,000 population in the West of Scotland. (1987)

Based on registrations received before April 1990.

(West of Scotland Cancer Surveillance Unit,1990)

Figure 1 : Fate of 100 patients with colorectal carcinoma



BLOOD SUPPLY TO THE LIVER

The normal liver has a dual blood supply via the portal vein (which collects blood from the viscera;the stomach,spleen,intestine and pancreas) and the hepatic artery which delivers blood directly from the heart.Both vessels enter the porta hepatis separately, before dividing into branches to the right and left lobes. (Rappaport,1973).

The separation of the liver into right and left lobes on the basis of surface anatomy does not fully reflect the vascular anatomy. Eight segments,each with separate hepatic arterial,portal venous and biliary radicles,may be identified. The hepar may therefore be divided more correctly into right (segments 5,6,7,8) and left(segments 2,3,4) livers,the line of separation corresponding to the plane from the gallbladder fossa to the inferior vena cava. Segment 1 (the caudate lobe) is considered to be a "third liver" with independent vascular and biliary anatomy (Couinaud,1957;Nakamura and Tsukuki,1981;Bismuth,1982).

The division of the portal venous and hepatic arterial radicles continues, until they unite at the origin of the hepatic sinusoids. Differences in pressure are thought to be compensated by sphincters(Rappaport,1973). There are also presinusoidal communications through which 30% of the arterial blood is shunted into the portal venous radicles before ultimately entering the sinusoids. These sinusoids are intimately associated with the hepatic parenchymal cells. Blood having passed through the sinusoids drain into hepatic venules that progressively coalesce to form the hepatic veins that ultimately drain into the inferior vena cava. The liver receives a quarter of the cardiac output,one third via the hepatic artery the remaining two-thirds are supplied by blood draining the splanchnic circulation into the portal vein(Lautt and Greenway,1987).

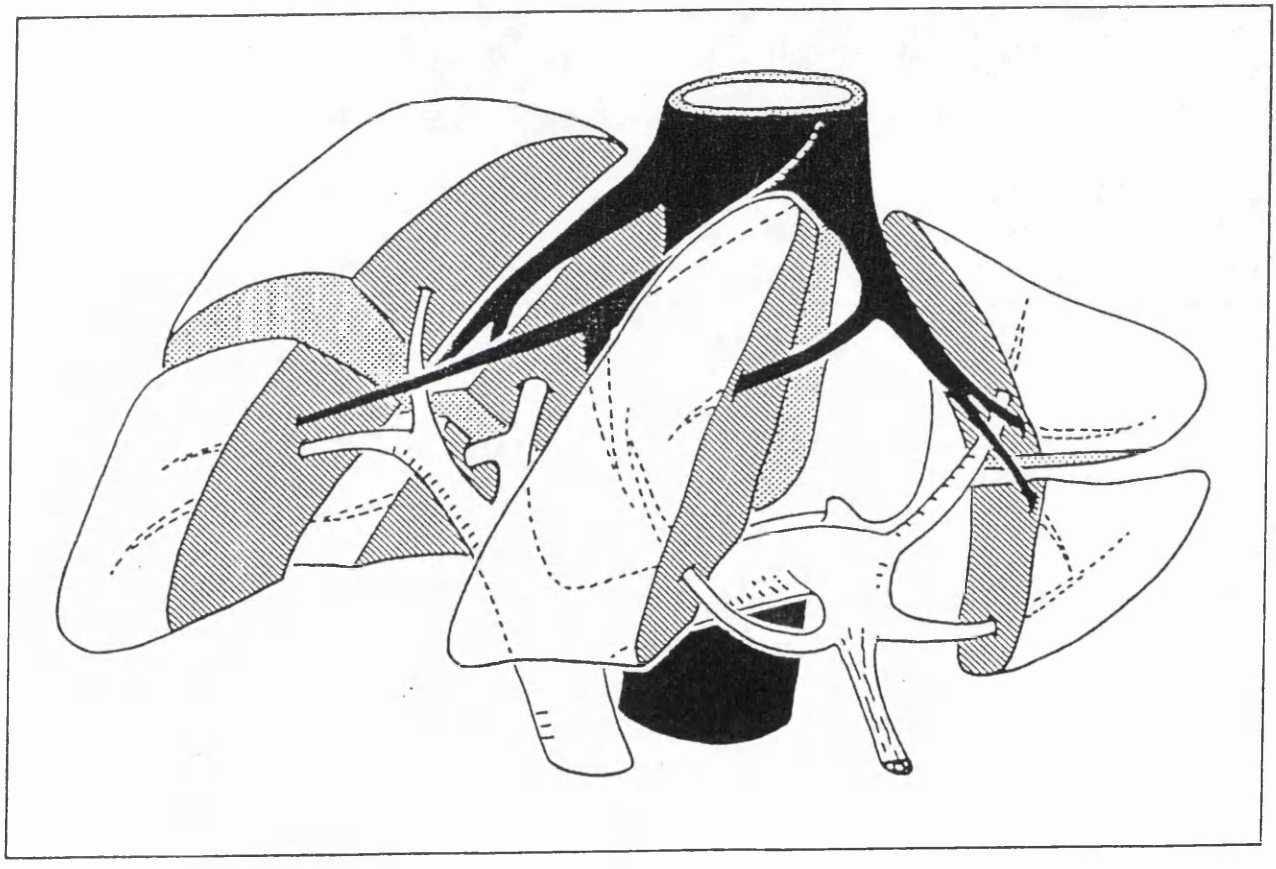


Figure 2: Segmental anatomy of the liver

Total liver blood flow has been estimated at approximately 1500 ml/min using colloidal techniques,BSP,indocyanine green and galactose clearance (Keiding,1988;Caesar et al,1961,Shaldon et al,1961; Bradley et al,1945). The flow within the liver is a function of the hepatic arterial vascular resistance and the portal vascular resistance. Alteration to systemic venous pressure does not appear to affect relative flow(Lautt and Greenway,1987). The resistance offered by the splanchnic vascular bed far exceeds that of the normal liver and the splanchnic bed therefore has the predominant effect on portal venous flow. Intrahepatic control of flow is therefore limited to the hepatic artery which is under both neural and humoral influences(Greenway and Stark,1971;Lautt and Greenway, 1987).

It has been shown in dogs that a decrease in portal venous flow leads to a compensatory increase in hepatic arterial flow. Similarly an increase in portal flow leads to a decrease in arterial flow,a feature that is enhanced in portal hypertension(Baker and Shields,1974;Zimmon and Kessler,1980). The mechanism remains unclear, but the present consensus is that these changes in arterial flow serve to buffer the homeostatic environment of the liver(Lautt and Greenway,1987) although the hepatic artery cannot always compensate quantitatively for reductions in portal flow.(Greenway and Stark,1971)

BLOOD SUPPLY TO LIVER METASTASES

Morphological studies on the structure of blood vessels that nourish hepatic metastases suggest that they are small tortuous vessels,arranged in a haphazard manner principally around the periphery of the deposit(Ackerman and Hechmer,1978). They are also immature lacking smooth muscle elements,endothelial tight junctions etc. and are therefore different from the surrounding host vessels in terms of physiological and pharmacological activity(Mattson et al,1977;Mattson et al,1978). Determining the source of the blood entering these vessels is important for the regional delivery of chemotherapeutic agents,to ensure maximum delivery to tumour cells.

There have been many methods used to determine the relative importance of the hepatic arterial and portal venous components of hepatic metastatic blood flow. These include the delineation of the vascular tree using barium sulphate(Segall,1923),India ink (Breddis and Young,1954) and other colloidal dyes(Fisher et al,1961), corrosion casting methods(Healey,1965;Lien and Ackerman,1970; Ackerman,1974),radioactive marker or stable isotope clearance techniques (Taylor et al,1979(^{133}Xenon);Ridge et al,1987(^{13}N),arteriography (Kido, 1970),and radio-active microsphere methods(Ackerman et al,1969;Ackerman, 1972;Hemingway et al,1991).

Micrometastases appear to have a dual supply,the portal supply being initially more prominent as in surrounding parenchyma. With growth the hepatic arterial contribution increases until the arterio-portal ratio is reversed, and in animal models tumours as small as 0.5mm have an established internal vasculature perfused predominately by the hepatic artery (Archer and Gray,1989). Once

established, human colorectal metastases would appear to be hypovascular relative to the surrounding liver parenchyma(Taylor et al,1978),although vascularity may vary between synchronous metastases(Bledin et al,1982;Civellari et al,1991;Daly et al,1985).

Histologically identifiable blood conduits are limited to the periphery of the metastases,central supply being haphazard and sparse resulting in ischaemic necrosis. In the physiological situation the portal contribution is minimal, a collateral circulation exists permitting portal venous filling of these vessels following arterial occlusion(Lien and Ackerman,1970). Despite the existence of these collateral vessels,radiolabelled macro-aggregated albumen microsphere experiments have failed to show significant shunts between the hepatic artery and hepatic veins in human colorectal carcinoma(Goldberg et al,1987).

In summary,human hepatic metastases from colorectal primaries appear to derive their blood supply almost entirely from the hepatic artery,are predominately hypovascular relative to surrounding liver parenchyma,and are associated with minimal arteriovenous shunting.

CONTROL OF INTESTINAL BLOOD FLOW

Intestinal blood flow is determined by three principal mechanisms: local myogenic or metabolic responses,sympathetic nervous control, and the effect of circulating vasoactive agents. Although discussed separately,these interact to maintain the homeostatic environment of the intestine.

The immediate control of flow is determined principally by local mechanisms largely independent of neural control(Johnson, 1964). In practice,phenomena resulting in a failure of the circulation to meet the O_2 demands of the tissues produce the formation of metabolites responsible for arteriolar vasodilation, with a consequent increase in blood flow.Potassium, H^+,serum osmolality,adenosine and adenine nucleotides,in addition to interstitial pO_2, have all been proposed as the local mediator(Granger et al,1980). Myogenic influences are dependent on the effect of stretch on vascular smooth muscle(Bayliss,1902), increases in transmural pressure leading to arteriolar vasoconstriction and reduced flow.

It is evident from this that there is considerable autoregulation of the intestinal circulatory environment. The principal site of this would appear to be the precapillary arterioles((Johnson and Hanson,1962). The extent to which the intestine is able to maintain constant flow is limited,flow reducing markedly when perfusion pressure falls below physiological values.The intestine is richly innervated by post-ganglionic vasoconstrictor fibres reaching the gut via the splanchnic nerves, stimulation of which reduces blood flow to the intestine(Bunch,1899). Continued stimulation results in a compensatory metabolic vasodilatation resulting in "autoregulatory escape"(Folkow et al,1964). Stimulation of parasympathetic nerves has little direct effect on intestinal blood flow,in mammals its action being principally on gut motility.(Kewentner,1965).

Numerous hormones alter intestinal blood flow either by directly affecting the resistance vessels or indirectly by affecting the perfusion pressure,sympathetic drive or by altering the local metabolic environment. Table 2 summarises the effect of hormonal stimulation on intestinal blood flow.

The most significant alteration to intestinal blood flow occurs with the response to food - the functional hyperaemia. Within a few minutes of ingestion splanchnic vascular resistance decreases,flow increasing by more than 100%(Burns and Worthington, 1969). This hyperaemia appears to be as a result of a combination of neural,hormonal,myogenic and metabolic stimuli the precise interaction of which remains the subject of much research.

Table 2 : Effect of hormonal stimulation on splanchnic vascular resistance and blood flow(Granger et al,1980).

Hormone	Vascular Resistance	Splanchnic blood flow
Glucagon	-	+
Prostacyclin	-	+
Gastrin	-	+
Secretin	-	+
CCK/PZ	-	+
VIP	-	+
Bradykinin	-(minimal)	?
5-HT	+(minimal)	?
Dopamine	+	-
PG A_2	+	-
Acetylcholine	+	-
Histamine	+	-
Vasopressin	+	-
Phenylephrine	+	-
Angiotensin	+	-
Noradrenaline	+	-
Adrenaline	+	-

METHODS OF DETECTING HEPATIC METASTASES

In the last decade,as evidence has increased that the ultimate survival in patients with colorectal metastases may be modified by surgery or chemotherapy,the detection of metastatic disease has assumed greater clinical importance. The simple detection of liver metastases is no longer sufficient,the evaluation of size,number and location may also affect future management.

Clinical manifestations of colorectal metastases,such as hepatomegaly, pain,weight loss or icterus occur relatively late in the clinical course,and are therefore of little use in detecting patients at a stage where they are still amenable to treatment. There are no specific tumour markers for colorectal tumours or their metastases. Carcinoembryonic Antigen(CEA) is disappointingly insensitive as a screening tool, but may have a role in the post-operative monitoring of metastases negative patients to detect preclinical recurrence.(Northover,1986;Wood et al,1980). The measurement of biochemical indices(Aspartate transaminase(AST), Alanine transaminase(ALT),gamma-Glutamyl transpeptidase(gamma-GT),Alkaline phosphatase) is of limited value in the detection of metastatic disease.(Jonsson et al,1984;Schreve et al,1984;Hugieur and Lacaine,1981;Kemeny et al,1982)

Metastases may be confirmed at laparotomy by palpation allowing biopsy. Alternatively,they may detected by imaging techniques which may be separated into two types. Firstly there are those that depend on a difference between the composition or density of the normal liver parenchyma compared to that of the abnormal lesion.

The four investigations of this type are computerised tomography (CT),real time ultrasonography(US),static hepatic scintigraphy,and magnetic resonance imaging(MRI),although MRI is still within Britain principally a research tool. Secondly there are those that attempt to exploit haemodynamic changes that occur in the presence of colorectal metastases. These changes may be determined by dynamic hepatic scintigraphy or by Doppler flow studies. Both investigations have yet to be accepted as being clinically relevant.

SECTION I - DENSITY DEPENDENT METHODS:

Real time ultrasonography was first developed in 1966,by Donaldson,an obstetrician in Glasgow;computerised tomography and albumen static scintigraphy were developed in the 1970's and MRI in the 1980's. The technology involved in these techniques has progressed rapidly in the intervening years,improving the precision of diagnostic imaging of hepatic metastases,and casting doubt on the relevance of studies of sensitivity and specificity performed in their infancy.(table 3). In all density dependent methods, detection may be complicated by the presence of diffuse abnormalities of the liver parenchyma,such as cirrhosis or fatty change.

Ultrasonography:

Real time ultrasonography depends on the detection of differences in the selective amplification of low level echos from an ultrasonic source directed at an organ. Metastases are detected due to a variations in the velocity of sound transmission through the normally homeogeneous liver parenchyma (Bryan et al,1977). Although with modern equipment lesions between 1 and 2cm can be diagnosed with confidence, and differentiation between solid and cystic lesions

readily made, a negative scan does not exclude metastases and the sensitivity of ultrasonography remains low,falling below 80% under study conditions(Kemeny et al,1982).Up to 10% of scans may be technically inadequate due to restricted anatomical access or overlying bowel gas(Schreve et al,1984). Another major drawback of sonography is its operator dependency,and in clinical practice accuracy has been reported to be as low as 20%(Charnley et al,1988).

Intraoperative Ultrasonography:

Percutaneous ultrasonography is hampered by the existence of "visually dead" areas due to the costal margin,bowel gas or simple obesity. The development of small,easily sterilised,high frequency ultrasound probes had led to the intraoperative examination of the liver which allows a more complete examination of the liver,with improved detection rates for intrahepatic lesions,especially in cirrhotic patients(Igawa et al,1985).

Originally described in 1980(Lane and Glazer,1980),several authors have claimed this to be the most effective method of detecting liver tumours(Igawa et al,1985;Gossetti et al,1985) detecting over 90% of liver metastases. The technique is time consuming and may require a "water cushion" to detect superficial lesions,although this may be considered unnecessary as these are easily detected manually.

Any test that takes intraoperative time to perform must potentially result in alterations in management to justify the effort. The detection and localisation of additional lesions in patients with multiple bilobular metastases is therefore of little interest. The possibility of detecting metastases in patients in whom all other investigations have been negative has been suggested(Gossetti et al,1985),although the efficacy of screening apparently clear livers by intraoperative

ultrasonography is yet to be fully established,as authors have tended to report the sensitivity in patients known to have intrahepatic lesions. Occasionally intraoperative ultrasound may be of benefit in distinguishing "Pseudotumours",such as focal hyperplasia in patients with coexistent hepatic parenchymal disease(Clain et al,1984)

The principle role of intraoperative ultrasonography would appear to be in patients in whom a liver resection is being contemplated. Currently only solitary lesions or less than three lesions localised to one area of the liver are considered suitable for resection. In these patients intraoperative ultrasonography has a dual role. The detection of previously occult lesions may render the patient an unsuitable candidate,avoiding an unnecessary resection. In addition by delineating the spacial relationship of the lesion to the principle intrahepatic vascular and biliary radicles,segmental resection may be performed with greater safety.

Computerised tomography

CT is a well established modality for the detection of liver metastases, the optimal technique having evolved with advances in scanning time and improved understanding of contrast media pharmacology. Early scanners required prolonged scanning times necessitating iodine infusions to prevent the rapid intravascular clearance of contrast media.Rapid scanning allows arterial or portal phase enhancement with improved detection, CT portography having the highest sensitivity in metastatic detection(Rossi et al, 1981).Bolus dynamic CT,CT arteriography and delayed iodine scanning, may also increase the information obtained over standard techniques.(Halvorson and Thompson,1991)

The detection of liver lesions is again dependent on the difference in attenuation or density between the background liver parenchyma and each lesion. Consequently isodense lesions,or lesions in a liver rendered of low density secondary to fatty change,are more difficult to detect.CT remains the most accurate currently available imaging technique for the detection of liver metastases with a sensitivity of up to 90%(Alderson et al,1983; Rossi et al,1981;Halvorson and Thompson,1991).

Magnetic Resonance Imaging

The spinning charges in an atom,the protons and electrons, convey magnetic properties to that atom.In general particles are paired in such a way that their spins cancel and materials with an even number of particles exhibit very weak magnetic properties. Magnets are a special class of materials in which the atoms contain several unpaired particles,giving these permanent magnetism.

The atoms of most importance for magnetic resonance imaging are those of low atomic number,having unpaired spinning particles within the nucleus. Each nucleus behaves like a small magnet,but as a whole lack columnation,the tissue having a neutral magnetic effect. By applying magnetic energy across the tissue,these nuclei can be orientated. On release from the external magnetic field,the orientation of the nuclei again becomes random, releasing energy. The amount of energy is a measure of the number of charged nuclei present and the rate of decay gives information about their environment. The release of this energy forms the basis of magnetic resonance imaging(Dendy and Heaton,1987).

Consequently by measuring the magnitude and kinetics of energy emanating from tissue exposed to and then released from an external magnetic field,a tomographic image can be created,providing anatomical detail. Thus the difference between liver parenchymal tissue and tumour tissue may allow identification of a colorectal metastatic deposit.The role of MRI in the detection of liver masses remains controversial. Respiratory artifact limits the application of MRI in some older machines. Stark's results (Stark et al,1987) suggested that MRI was superior to CT in the detection of metastases. In this study,he compared a state of the art MRI scanner to older CT equipment. By contrast other authors have found CT to be consistently superior to MRI(Nelson et al,1988).

In Britain,the limited availability of MRI has prevented its inclusion into clinical practice,and at present MRI remains a research tool. The sensitivity and specificity of CT and MRI with modern equipment are probably similar, these investigations being complementary rather than mutually exclusive.

Static scintigraphy

The detection of liver metastases by scintigraphy relies on the ability of the normal liver parenchyma to concentrate systemically administered ^{99m}Tc labelled albumen colloid,metastases resulting in defects in the hepatic image.Hepatic scintigraphy is feasible in nearly all patients,and requires minimal medical input (Bryan et al,1977) and it is therefore a particularly useful screening tool when resources are limited. Scintigraphy is associated with a relatively high false positive rate due to large pendulous breasts,intrahepatic gallbladder,renal indentations,or lesions close to the porta hepatis(Knopf et al,1982). Scintigraphy also provides physiological information on liver function,but anatomic definition is poor and positive findings require corroboration.

SECTION II - HAEMODYNAMIC TECHNIQUES:

Changes in hepatic blood flow with the development of intrahepatic tumour:

Accurate measurement of accurate organ blood is difficult in the clinical situation,and classical techniques for measuring liver blood flow fail to distinguish between the hepatic arterial and portal venous components. This has led to a dependence on the findings from animal studies. The knowledge that hepatic metastases derived the majority of their blood supply from the hepatic artery ,coupled with the observation that the hepatic artery contributes a greater proportion of total liver blood flow(as determined in the hepatic perfusion index) in the presence of hepatic metastases, led to the assumption that absolute hepatic arterial flow increased with the development of metastases(Boyd et al,1978;Leveson et al,1985;Sarper et al,1981).

Using the Walker cell model of colorectal metastases,using radioactive microspheres and electromagnetic flowmetry,Nott demonstrated that this fundamental assumption was wrong,and that although the HPI increases,this is due to a decrease in portal venous flow rather than increased arterial demand(Nott et al,1989). He suggested that the reduction in portal venous inflow was secondary to the development of significant arteriovenous shunts,increasing portal venous resistance,and therefore reducing flow.

Human metastases are slow growing,generally hypovascular,and are not associated with significant systemic shunting,and consequently, this model has been criticised in that it is fast growing,hypervascular and exhibits significant arteriosystemic shunting. Hemingway,using the hypovascular HSN sarcoma model, which is more representative of human colorectal metastases, confirmed the that portal venous inflow fell and hepatic arterial inflow remained static with the

development of intrahepatic tumour(Hemingway et al,1991).In contrast to the Walker cell model,these haemodynamic changes occur in the HSN model without the development of arteriovenous shunts,the portal venous resistance being unchanged. This suggested that the reduction in portal venous flow must be due to an increase in splanchnic vascular resistance,with a consequent reduction in splanchnic vascular, and therefore, portal flow.

As described earlier the control of splanchnic vascular resistance and therefore blood flow is multifactorial. Although immediate control would appear to be as a result of local mechanisms,persistent alterations in the haemodynamic pattern of the splanchnic bed appear to result from a combination of humoral and neural control. Many tumour products are vasoactive,however,if the tumour is producing an active substance why are the changes only apparent with the development of intrahepatic tumour? Although the accuracy of measuring blood flow using duplex sonography has yet to be confirmed ,early results suggest that the changes may be due to a combination of reduced splanchnic vascular flow and increased hepatic arterial flow but the numbers investigated are as yet small(Leen et al,1991).

In summary it would appear that with the development of metastases,total liver blood flow does not increase and may even fall,primarily due to a reduction in portal venous flow which is partially compensated for by a minor increase in arterial inflow.

Dynamic hepatic scintigraphy

In 1981 Sarper and Biersack described techniques whereby they could distinguish the arterial and portal phases of liver blood flow by the dynamic analysis of liver and right renal images following in a bolus injection of ^{99m}Tc sulphur colloid(Biersack et al,1981;Sarper et al,1981). In this technique,posterior images are obtained every 2 seconds for 2 minutes,using a large field of view gamma camera. Images corresponding to the heart and lungs,liver,spleen and kidneys may be identified. By the creation of time activity curves,blood flow to each organ can be deduced from the rate of delivery of ^{99m}Tc sulphur colloid to that organ.

Analysis of the renal time activity curve reveals a single peak, corresponding to colloid reaching the kidney via the renal artery, followed by a gradual fall as colloid passes into the venous circulation. By contrast the hepatic time activity curve has two phases of uptake,an initial phase corresponding to hepatic arterial delivery of colloid and a second steeper phase due to colloid reaching the liver having initially passed through the splanchnic vascular bed. The end of the arterial phase and start of the portal phase may be determined as the point at which counts over the reference organ(kidney or spleen) are maximal.

Initially the right renal image was used as a reference organ,but the technique was modified by Wraight in 1982 using a splenic image to mimic arterial flow(Wraight et al,1982). The following year Parkin,in Leeds,used the left renal peak for analysis to avoid hepatic overlap with the right renal image,which results in artificially high values(Perkins et al,1987),and described the ratio of the contribution of the hepatic arterial flow to total liver blood flow as the " Hepatic Perfusion Index".

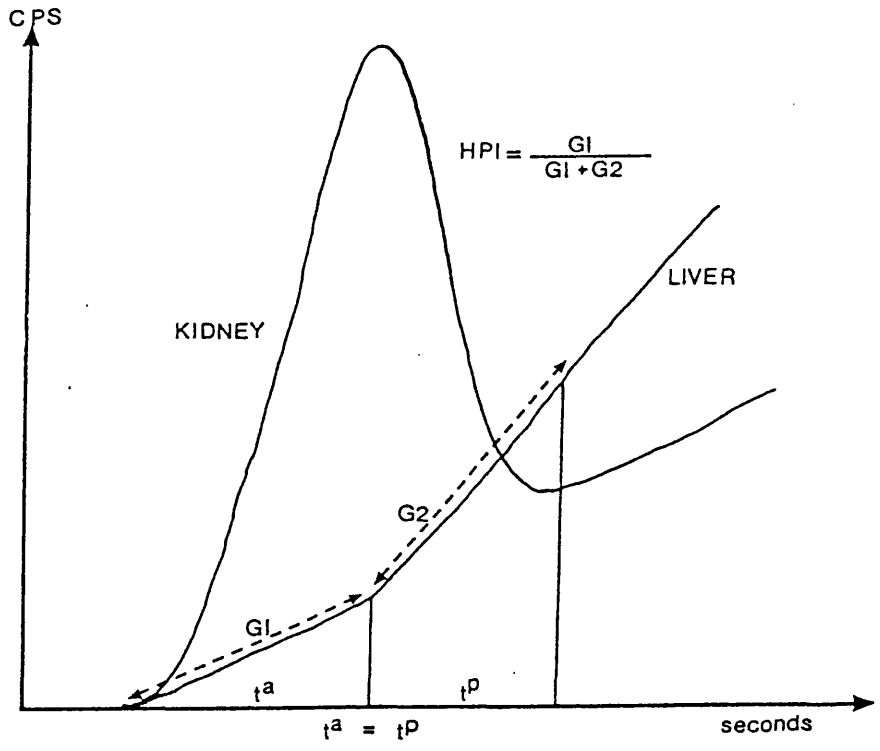


Figure 3: Time activity curves of liver and left kidney following an intravenous bolus of ^{99m}Tc albumen colloid.

The Leeds group showed that 97% of patients with metastases proven at subsequent laparotomy had an abnormal preoperative HPI (Parkin et al,1983). Some apparently metastases-free patients had an abnormal HPI at the time of laparotomy.Follow-up of these patients confirmed that at one year 18 of 29 patients with an abnormal HPI had developed metastases,whereas none of 21 with a normal pre-operative HPI had gone on to develop metastases(Leveson et al,1985). Subsequently,the ability of the HPI to predict the metachronous development of metastatic disease was confirmed(Cooke et al,1987), and the HPI was also shown to increase on serial scanning with disease progression in 86% of patients.(Ballantyne et al,1990)

Despite these encouraging and potentially clinically important results,the hepatic perfusion index(low administered activity(80 MBq)),has not been accepted into surgical practice. This has been as a result of other workers experiencing technical difficulties or problems with the reproducibility of the technique. Some authors have found up to 14% of patients unsuitable for analysis,although this may be reduced using the left kidney image for analysis(Perkins et al,1987). Laird confirmed the HPI to be a sensitive method of detection of liver dysfunction,but found abnormalities in patients with parenchymal liver disease,in addition to those with metastases (Laird et al,1987).

The standard technique involves the injection of 80-100 MBq of ^{99m}Tc. This produces relatively small counts in each region of interest,and previous studies from the Glasgow Royal Infirmary have demonstrated a range of values on reprocessing studies by a single observer(Goldberg et al,1989). This is because the inherent statistical errors associated with small accumulated counts lead to relatively poorly defined uptake curves.

In addition to this intra-observer variability,Perkins reported that significant interobserver variation occured on sequential analysis of time activity curves(Perkins et al,1987),with one observer consistently achieving higher results than another,possibly due to differences in the size of regions of interest selected.

Standardised methodology is of paramount importance in that bolus quality,the length of time over which the slopes are averaged,transit times through the liver,splenic and mesenteric circulations and the degree of tracer extraction will all effect the calculated HPI(Tindale and Barber,1987). Errors are most likely within the abnormal range,and higher values are obtained using the right rather than the left renal image(Perkins et al,1987). We have previously found that reliability was improved in animal studies,measuring the HPI using a high administered activity of radiocolloid(80 - 100MBq/rat)(Hemingway et al,1991). This has led to the evaluation of the Hepatic Perfusion Index in patients using a high administered activity of radiocolloid(400MBq / patient) in an attempt to improve the reproducibility of the technique,and the results of this assessment are presented later in this thesis.

Duplex ultrasonography

More recently advances in the technology of duplex sonography are being applied in Glasgow Royal Infirmary as a technique for the estimation of hepatic arterial and portal venous flow(Leen et al,1991). The technique is less invasive than dynamic scintigraphy and again allows the calculation of a doppler perfusion index,defined in analogy with the hepatic perfusion index as the ratio of the hepatic arterial inflow to total liver blood flow. The inter- and intra-observer variation and clinical correlation have yet to be evaluated. Another potential advantage of Doppler sonography is the additional information that may be obtained regarding the morphology of the apparently normal liver parenchyma. False positive HPI's occur in the presence of cirrhosis (Parkin et al,1983) which may be detectable on ultrasound. This allows the calculation of a "congestive index" which compensates for other morbid conditions.

Summary:

Over the last 15 years,there have been many published studies examining the relative sensitivity of various methods of detection of liver metastases (table 3). Imaging technology is an area of rapid evolution, rendering sensitivities obtained on 1970's equipment irrelevant. All techniques, and in particular ultrasound and doppler perfusion scanning,have an element of operator dependance,techniques such as dynamic scintigraphy being dependant more on computer analysis rather than a subjective impression. Finally, most reports on new imaging techniques are by protagonists,and therefore have a tendency toward subconscious bias,and a techniques clinical application is determined by its accuracy in the hands of those less familiar with its use.

It would appear that not only is dynamic scintigraphy the most sensitive technique for the detection of colorectal metastases, consistently having a sensitivity in excess of 90%(Cooke et al,1987;Sarper et al,1981;Parkin et al,1983;Leveson et al,1985),but in addition has the ability to provide prognostic information in patients undergoing apparently curative surgery. Consequently if problems of reproducibility are overcome the measurement of the HPI may have a central role in the investigation and management strategy of patients with colorectal carcinoma.

Table 3: Accuracy of methods of detecting hepatic metastases.

	Sensitivity of detection techniques(%)							
	Static	**U/S**	**CT**	**Palp**	**MRI**	**HPI**	**Bioch**	**IOU/S**
Bryan(1977)	79	85	88				30	
Sarper(1981)85						100		
Finlay(1982)	29	47	88	30				
Kemeny(1982)	82	75	80				65	
Alderson(1983)	86	82	93					
Parkin(1983)						97		
Schreve(1984)	79	85	88				30	
Igawa(1985)								91
Gossetti(1985)		80	74					98
Leveson(1985)						94		
Stark(1987)			51		64			
Nelson(1988)			86		62			
Charnley(1988)	21	50	64					86

METHODS OF TREATING METASTASES.

Eighty percent of patients that die of colorectal carcinoma have liver metastases at the time of death(Taylor et al,1985). In the absence of liver or lung metastases spread to other organs is rare. Treatment may be designed to prevent their occurrence (adjuvant therapy) or towards eradication or control of established disease. Chemotherapy or radiotherapy may be employed in either a therapeutic or adjuvant setting,whereas surgery is indicated only in the presence of established metastases.

(1) CHEMOTHERAPY:

SECTION I - TREATMENT of ADVANCED DISEASE

Systemic therapy:

Chemotherapy is the only feasible option when faced with a patient with advanced disease. Many feel that subjecting these patients to the morbidity associated with chemotherapy is unjustified when the median survival may be as short as 3 months(Wood et al,1976). Systemic 5FU is still the most effective agent producing objective responses in 10-20% of patients but does not affect survival(Kemeny,1987;Laurie et al,1989).Most regimes are limited by systemic toxicity.Consequently two alternative approaches toward improving the efficacy of chemotherapy have been employed: tumour targeting and biochemical modulation of 5FU activity.

TREATMENT TARGETING:

The targeting of therapy toward a tumour may be considered on three levels(Widder et al,1979):

(1) to the affected organ

(2) to the tumour mass within that organ

(3) to the tumour cells within the tumour mass

First Order targeting:

(a) Surgical methods of targeting;

In 1964 Watkins published and his collegues from Boston published their initial experience (Watkins and Sullivan,1964) with regional infusion of fluorodeoxyuridine into the hepatic artery; long term follow-up of these patients (Watkins et al,1970) suggested a favorable symptomatic response and also prolongation in survival when compared to historical controls. In the next 20 years several phase I and II trials of 5-FU,FUDR and mitomycin (Patt et al,1980;Ansfield et al,1977) reported objective response rates as high as 83% but the percutaneous catheter technique resulted in a high complication rate.

The development of an implantable port, through which intraarterial infusions could be administered (Shiley-Infusaid,1981) stimulated a further phase II trial of intra-arterial FUDR therapy which again reported an 83% response rate(Ensminger et al,1982). Unfortunately the phase III study that followed this allowed crossover following failure of systemic therapy preventing analysis of survival data(Holn et al,1989). A similar trial(Kemeny,1987) again showed improved hepatic response rates but again the cross-over design obscured survival data. Infusion therapy would appear to be more effective than interval bolus administration (Lokich et al,1989).

In both forms of therapy the limiting factor was patient tolerability;approximately double the maximum systemically tolerated dose(300mg/m^2/d)(Lokich et al,1989) was required to produce significant biliary morbidity in the intra arterial group. Nearly twice as many patients developed extrahepatic disease in the intraarterial group (62.5%:35%(Kemeny,1987)).

(b) Biochemical methods of targeting:

An alternative approach for first order targeting is to utilise inert drug carriers for site specific delivery to the target organ. Human albumen and dextrans(Baurain et al,1983) have been evaluated as potential carriers,but the potential immunogenicity limits their clinical usefulness. Consequently synthetic carriers, consisting of a polymer backbone to which can be linked both targeting residues and antitumour agents,have been suggested as potential alternatives.

The galactose recognising receptor of hepatocytes has been proposed to enable liver specific chemotherapy to be systemically administered.Previous *in-vitro* experiments using N-(2-hydroxylpropyl)methacrylamide copolymers containing galactosamine have confirmed that binding occurs to both hepatocytes and hepatoma cell lines(O'Hare et al,1989),and hepatic targeting occurs in murine distribution experiments. Little is known regarding its distribution in tumour bearing livers.

An alternative method of biochemical targeting to liver is to utilise porphyrin metabolism. When haemoglobin is broken down the "globin" portion is reutilised either as such or by joining the amino acid pool. The "haem" or porphyrin portion is

broken down and the Fe^{2+} removed to form biliverdin which is subsequently reduced to bilirubin. This occurs in the reticuloendothelial cells of the liver spleen and bone marrow. Exogenous porphyrins are metabolised in a similar manner,resulting in organ specific targeting to the liver and spleen.

An additional attraction of porphyrins is the spontaneous localisation in tumour tissue,first noticed by Policard in 1924. Haematoporhyrin derivative(Lipson et al,1961) and later dihaematoporphyrin ether/Photofrin II(Dougherty et al,1983) have been developed for their improved tumour localising properties. If a cell in the presence of a photosensitiser is stimulated,classically by light in the near infra red,a number of reactions occur, the most important of which is the production of singlet oxygen(Dougherty et al,1978) which results in membrane damage and cell death due to photooxygenation of membrane proteins(Van Steveninck et al,1986).

Consequently in porphyrins we have the potential for organ selectivity, tumour localisation and local cytotoxicity. Unfortunately the poor tissue penetration of visible light(5mm at 630nm)coupled with the inaccessability of the liver prevent standard photodynamic therapy having a clinical application for the treatment of liver metastases. In theory any non-ionising electromagnetic radiation has the potential to excite a photosensitiser, raising the possibility of intrahepatic excitation with external beam radiation. This innovative approach is as yet theoretical.

The principal advantage of biochemical targeting by either galactose bearing polymers or porphyrins is the avoidance of major surgery with its attendant morbidity and mortality. At present this alternative strategy remains in its infancy.

Second order targeting:

Although first order targeting reduces the systemic morbidity and allows modest dose escalation,colorectal hepatic metastases are relatively hypovascular relative to the surrounding liver parenchyma, favoring drug delivery to normal liver.The development of significant hepatotoxicity prevents dose escalation to achieve cytotoxic levels within the tumour deposits.

Tumour vessels are immature and,lacking smooth muscle elements (Mattson et al,1977;Mattson et al,1978),are unable to respond to vasoactive agents. Any response following administration of a vasoconstrictor will occur predominately in the normal liver parenchyma inducing a temporary tumour hypervacularity,favoring delivery of drug to tumour rather than liver. The use of biodegradable emboli such as albumen or starch microspheres have also been shown to increase the retention of drug in tumour tissue(Cooke and Chang,1990). The temporary embolisation within the liver delays the systemic distribution and leading to reduced systemic exposure due to a prolonged first pass effect(Lindell et al,1978).

Both vasoactive agents and degradable emboli have been used clinically(Civellari et al,1991;Goldberg et al,1989;Hunt et al,1990),to produce second order targeting of chemotherapeutic agents. Excellent objective responses have been achieved,but this has as yet to be shown to improve survival.

The available evidence would suggest that while first and second order targeting may produce symptomatic and objective intra-hepatic responses, any survival advantage is limited by the subsequent development of extrahepatic disease. Titration of the intrahepatic dose to allow sufficient spillover into the systemic circulation to achieve adequate systemic levels is at present being investigated.

Biochemical enhancement of 5-FU activity

Because of the relative insensitivity of colorectal metastatic disease to all currently available chemotherapeutic agents,there has recently been considerable interest in the potentiation of the achieved antitumour effect through biochemical modulation. Experimental studies investigating the mechanism of action of 5-FU(Evans et al,1981;Waxman and Bruckner,1982) have suggested that the inhibition of thymidylate synthetase is one of the major anti-metabolic sites of action of the drug. The duration of inhibition appears to depend on the 5-FU metabolite,flourodeoxyuridylate, forming a ternary complex with thymidylate synthetase and the folate cofactor 5-10 methylene tetrahydrofolate. Other folate

Table 4: Trials of 5-FU plus Folinic acid versus 5-FU alone.

Erlichman (1988) :	Improved response rate
	Prolonged disease free interval
	Prolonged survival
Poon (1989):	Response rate best with low dose FA
	Prolonged disease free interval
	Prolonged survival
Doroshaw (1989) :	Improved response rate
	Prolonged disease free interval
	? trend toward prolonged survival
Petrelli (1989)	Response rate best with high dose FA
	? trend towards prolonged survival

analogues also stimulate the binding of flourodeoxyuridylate to thymidylate synthetase. The addition of folinic acid,is thought to increase or substitute for 5-10 methylene tetrahydrofolate,so that more ternary complexes may develop producing an anti-metabolic effect of greater magnitude.

Encouraging results from phase I and II studies(Machover et al,1986;Madajewicz et al,1984) of combination therapy,were confirmed in larger comparative trials (Erlichman,1988,Doroshaw et al,1990) both in terms of objective responses and survival. Studies investigating the effect of folinic acid dose escalation produced conflicting results, Petrelli(Petrelli et al,1989) suggesting dose escalation to be beneficial, while Poon (Poon et al,1989) suggested this made little difference. Both trials confirmed that the addition of folinic acid potentiated the response over 5-FU alone.

An alternative pathway for biochemical modulation is to use PALA(N-Phosphonacetyl-L-aspartate) which is an inhibitor of aspartate transcarbamylase, an essential enzyme in pyrimidine synthesis.This indirectly results in increased activation of 5-FU to 5-fluorouridine triphosphate resulting in increased RNA incorporation. Early clinical trials have suggested response rates in excess of 40% may occur with this regime((Ardalan et al,1988; O'Dwyer et al,1989). Combination of 5-FU with methotrexate appears to act in a similar manner(Marsh et al,1989). Other combinations with cisplatin or dipyridamole are in their infancy. The improved understanding of the mechanism of action of these agents suggests further advances will be made in the treatment of these relatively chemoresistant tumours.

SECTION II

TREATMENT OF MICROMETASTATIC DISEASE(Adjuvant therapy):

(1) Systemic:

Adjuvant systemic therapy is designed to prevent recurrent disease developing in patients thought to have had a curative resection,by treating the patients with minimal tumour bulk at the micro-metastatic stage. A recent review of 27 randomised trials of adjuvant chemotherapy suggested that whereas the majority of regimes had little effect,those containing 5FU as the principal agent resulted in a small benefit in terms of survival(Buyse et al,1988).

An additional approach is the addition of the immunomodulator levamisole,however the initial study from Glasgow showed no benefit(Bancewicz et al,1980). Larger studies have confirmed that levamisole is of no benefit when used alone(Arnaud et al,1989), but it may be of benefit when used in combination with 5-FU.Three major studies (Windle et al,1985;Laurie et al,1989; Moertel et al,1990) involving 1700 patients with stage B or C carcinoma of the colon, have shown that a significant improvement in survival can be achieved using adjuvant therapy with combination 5-FU and levamisole,although the benefit appears to be limited to those patients with lymph node involvement.

(2)Regional:

Between 10 and 30 percent of patients undergoing radical resections for carcinoma subsequently develop liver metastases (Russell et al,1984;Finlay and McArdle,1982),and of these a third are apparently confined to the liver. These presumably arise by haematogenous spread along the portal vein. In an attempt to reduce the morbidity associated with chemotherapy,and target the drug to the liver,short term portal venous infusion of 5-FU has been used with minimal toxicity. In a prospective randomised trial Taylor reported a significant improvement in survival in patients treated with postoperative portal venous infusion. The benefit was limited on retrospective analysis to subgroups within the study cohort(Taylor et al,1985). Several interim reports of other studies have been less conclusive. The AXIS (Adjuvant Xray and 5-FU Infusion Study)is currently addressing this controversy.

In summary,some progress has been made in the treatment of advanced colorectal carcinoma and in the adjuvant setting. Of the many drugs have been tried over the last 30 years,5-FU remains the mainstay of treatment.The most promising results would appear to be achieved using 5-FU in combination with a biochemical or immunological modulator,rather than in combination with other cytotoxics. Objective responses and improvements in survival are possible in patients with advanced disease, adjuvant therapy appears to be more promising as regards the potential for long term survival. Systemic or host organ toxicity remains a major problem limiting the administered dose. Identifying the "at risk" population and improving the targeting of treatment to minimise toxicity may provide improved results with available agents.

(2)RESECTION OF METASTASES

Resection of liver metastases was first performed by Garre over 100 years ago(Hunt et al,1990),however its role in current management remains controversial. Adson,1976;Cady and McDermott,1985). Resection in patients with solitary metastases or small multiple deposits in one area of the liver may improve survival,resulting in between a 25 - 40% five year survival rate with a less than 5% operative mortality rate(Adson et al,1984;Iwatsuki et al,1986). It must be remembered that there is potential for prolonged survival in patients with low volume disease without treatment(See table 5).

Table 5: Survival in patients with untreated potentially resectable metastases

	Type of metastases	Median Survival	% 5YS
Wood (1976)	"resectable"		8%
Pettavel (1978)	Solitary/small	22 mths	
Wanebo (1978)	solitary	19 mths	
Goslin (1982)	<4	24 mths	
Wagner (1984)	Solitary	21 mths	
Daly (1985)	< 20% PHR	>24 mths	

The development of more sophisticated imaging techniques has revealed an increasing number of patients with multifocal or extrahepatic disease, casting doubt as to whether any metastases are truly solitary.Adson showed that at least 50% of metastases thought to be solitary had synchronous lesions(Adson et al,1984) . Even with improvements in postoperative care perioperative mortality remains around 5 - 10%,(Ekberg et al,1986;Fortner,1988, Wanebo et al,1978),with morbidity rates as high as 25%(August et al,1985;Logan et al,1982), requiring a substantial improvement in survival to offset the risks of surgery. The recurrence rates following hepatic resection are also high,varying between 35 and 90%(Cady and McDermott,1985;August et al,1985;Joishy and Balasegaram,1980). The variation being at least partly as a result of small numbers in each study.

Table 6: Results of resection of liver metastases:

Author	No of patients	Recurrance
Joishy (1980)	10	90%
August (1985)	33	50%
Cady (1985)	18	35%
Rajpal(1982)*	30	50%
Fortner (1988)*	77	58%

* excluding operative mortality.

Correct patient selection is obviously of utmost importance and currently only solitary or up to three metastases localised to one area of the liver are considered suitable for resection. More extensive procedures are associated with significantly increased morbidity and have as yet to be shown to benefit the patients. Even if estimates that up to 10% of patients with metastases may benefit from resection are justified,this relates to a relatively small number of patients. In the U.K., thirteen thousand patients die as a result of colorectal cancer each year. Approximately 3750 will have metastases apparently confined to the liver at the time of death. Even if 10% (and the true figure is probably closer to 4%) of these are suitable, this relates to only 15 patients a year in the West of Scotland as being potential candidates for resection in the entire region.

Because 20-30% of the patients dying from colorectal cancer have metastatic disease apparently confined to the liver, hepatic transplantation has been suggested as a possible treatment. Starzl and his colleagues from Pittsburg have shown that transplantation has no advantage over resection,except in patients with end stage hepatic parenchymal disease(personal communication).

(3)HEPATIC ARTERY LIGATION

The rationale of hepatic arterial ligation stems from the observation that hepatic metastases derive their blood supply from the hepatic artery rather than the portal vein,while the normal hepatic parenchyma can withstand interruption of this supply. Reinhoff and Woods in 1953 described the effect of hepatic arterial ligation on metastases from an oesophageal primary. Nilsson (Nilsson and Zettergen,1967(1);Nilsson and Zettergen,1967(2)) demonstrated that hepatic arterial ligation was effective in reducing the size of experimental rodent hepatic tumours. There is some experimental evidence that distal embolisation rather than proximal ligation may be more effective (Doppman et al,1978).

In human colorectal tumours,despite evidence that ligation or embolisation leads to necrosis of the majority of tumour cells,with an objective response rate of up to 30% of patients(Chuang and Wallace,1981), the effect is temporary with the development of collateral channels within a week of surgery(Bengmark and Rosengren,1970), and the treatment has little effect on survival (Almersjo et al,1972). Bengmark and his colleagues from Lund introduced an implantable inflatable cuff to permit intermittent hepatic arterial occlusion and have shown significant objective responses without the development of arterial collaterals. The combination of hepatic arterial ligation with intraportal chemotherapy as first described by Fortner (Fortner and Pahnke,1976),only produced objective response rates of 20% and no effect on survival (Gerard et al,1991).

(4)RADIOTHERAPY

Although colorectal adenocarcinoma is potentially radiosensitive,as suggested by evidence that adjuvant radiotherapy may reduce the rate of local recurrence in patients with rectal cancer,whether given preoperatively(Gerard et al,1985;S.R.C.G,1987) or post operatively(G.T.S.G,1985),its role in the treatment of advanced disease is limited by the local toxicity.

Palliation of abdominal pain,nausea and vomiting can be demonstrated in up to 90% of patients undergoing external radiotherapy for hepatic metastases(Sherman et al,1978;Borgelt et al,1981)but this treatment has not been associated with a prolongation of survival (Sherman et al,1978;Turek-Maischeider and Kazem,1975). If a dose exceeding 3500 rads is administered severe irreversible radiation hepatitis results.

The exposure of the normal hepatic parenchyma to radiation may be reduced by the regional delivery of glass microspheres, containing yttrium-90(^{90}Y). These are prepared as ^{89}Y,which is then activated by neutron bombardment to ^{90}Y, a beta emitter with a half life of 64 hrs. Each microsphere therefore irradiates the tissue surrounding it,with tissue penetration limited to less than 0.85mm. Herba showed that both symptomatic improvement and objective responses could be achieved in up to 60% of patients without significant hepatic morbidity(Herba et al,1988).

Following administration into the hepatic artery,microspheres are not distributed according to blood flow. Due to an unknown mechanism,there is preferential delivery toward tumour tissue(Cooke and Chang,1990),with consequent sparing of the normal hepatic parenchyma from the effects of the beta irradiation. This technique has been further refined in a recent phase 1 study in Glasgow Royal Infirmary, University Department of Surgery,combining regional delivery with angiotensin targeting.

This study has shown that up to 15,000 rads can be delivered to an individual patient,without the development of significant toxicity. Using this technique significant objective tumour responses have been achieved but financial and logistic difficulties limit the universal acceptance of this form of therapy.

AIMS OF THESIS

Colorectal carcinoma is a common clinical problem, and as such advances in this field have wide applications. This review of the literature has covered many aspects of the current methods of detection and management options for colorectal hepatic metastases,and has highlighted some of the areas in which improvements in treatment are being sought. It is clear that whatever treatment option is chosen,early accurate diagnosis of metastases,holds the key to effective treatment.

Current treatment options for patients with colorectal metastases are limited. In the majority of patients, cytotoxic therapy provides the only hope for improved survival.The benefit from chemotherapeutic regimes is greatest when the tumour load is small,treatment being given at a micrometastatic stage as an adjuvant therapy,however,given in this manner,more than half of those treated, will receive their chemotherapy unnecessarily.

Advancing technology has ensured that the accuracy of density dependent detection techniques has steadily improved over the last 15 years. There is a threshold below which metastases are undetectable leading to incorrect staging of patients. Methods dependent on the haemodynamic changes that occur in the presence of colorectal metastases hold great promise, as they may be able to detect micrometastatic spread of disease. The accuracy,reproducibility and mechanism underlying these changes has not yet been clarified.

Having identified a treatment population, the effect of current chemotherapy is limited by systemic toxicity. Hepatic targeting aims to increase the effective dose to tumour tissue while at the same time reduce systemic exposure. This has traditionally involved surgical catheter placement, however it may be possible to manipulate biochemical pathways to achieve tumour localisation.

In this thesis I have carried out two broad lines of investigation into the detection and treatment of liver metastases. The aims were as follows;

(1)to evaluate the reproducibility,the intra and interobserver variation of the high administered activity Hepatic Perfusion Index and prospectively compare the HPI with the other currently available imaging techniques for the detection of hepatic metastases.

(2)to evaluate the mechanisms that underlie the changes in the HPI with the development of hepatic tumour,and

(3)to evaluate potential methods of biochemically or pharmacologically targeting chemotherapeutic agents to a rodent model of colorectal metastases.

CHAPTER 2

METHODOLOGY

In this chapter I will describe details of the methods employed to investigate the aims of the thesis. In this, and in subsequent chapters,this is divided into three subsections:

(1) CLINICAL STUDIES:

Firstly I will describe the modifications in the methodology of the Hepatic Perfusion Index(HPI) made in Glasgow Royal Infirmary, and my subsequent evaluation of its reproducibility. I will then discuss the technique we have utilised for our intraoperative ultrasonography, followed by an assessment of how both techniques performed in a prospective clinical study of the detection of colorectal metastases.

(2)HAEMODYNAMIC STUDIES:

In the second section I will describe the methods involved in the production of the animal model of colorectal metastases used in this thesis. I will also describe the methods employed in experimental studies on the vascular changes that accompany the development of hepatic metastases. These were performed in an attempt to prove that the reduction in splanchnic vascular inflow and increase in splanchnic vascular resistance was due to a humoral and therefore transmissible vasoactive agent. Two experiments were performed; a cross perfusion study and a reference microsphere study.

(3)TARGETING STUDIES

In this section, I will describe the experiments studying three potential methods of biochemically targeting the delivery of drugs to hepatic parenchymal tissue and lastly an experiment investigating how two methods of second order targeting may be combined to achieve the maximal response.

SECTION I - CLINICAL STUDIES

HEPATIC PERFUSION SCINTIGRAPHY(high administered activity)

Patients were fasted for 12 hours. The first description involved the injection of 300MBq of ^{99M}Tc sulphur colloid(Boyd et al,1978),the technique being modified to a "low administered activity" technique in the early 1980's,to minimise patient exposure. This involved the injection of 80 MBq(Perkins et al,1987),111MBq (Sarper et al,1981;Parkin et al,1983) or 150MBq (Wraight et al,1981),however,we have found a resultant reduction in image quality,due to poor count statistics, with a consequent increase in interobserver variation (Goldberg et al,1987).

In an attempt to improve the reproducibility, a modified "high administered activity" technique was employed,each patient receiving a rapid intravenous bolus injection of 400 MBq of ^{99m}Tc albumen colloid via the antecubital vein. Posterior images were obtained every 2 seconds on a 64 x 64 matrix using a large field of view gamma camera,fitted with a high sensitivity parallel hole collimator, interfaced with a computer. Regions of interest were drawn around the liver and both kidneys avoiding overlap with the lungs and aorta. Using the left kidney arterial peak (Tp) as the demarcation of the hepatic arterial and portal venous components of liver perfusion,the gradients of the eight second periods before(G_1) and after(G_2),but excluding the frames overlapping Tp,were calculated using a least squares analysis.

The Hepatic Perfusion Index(HPI) is therefore defined as the ratio between the hepatic arterial gradient(G_1) and the total liver blood flow(gradient $G_1 + G_2$).

$$\text{i.e. HPI} = \frac{G_1}{G_1 + G_2}$$

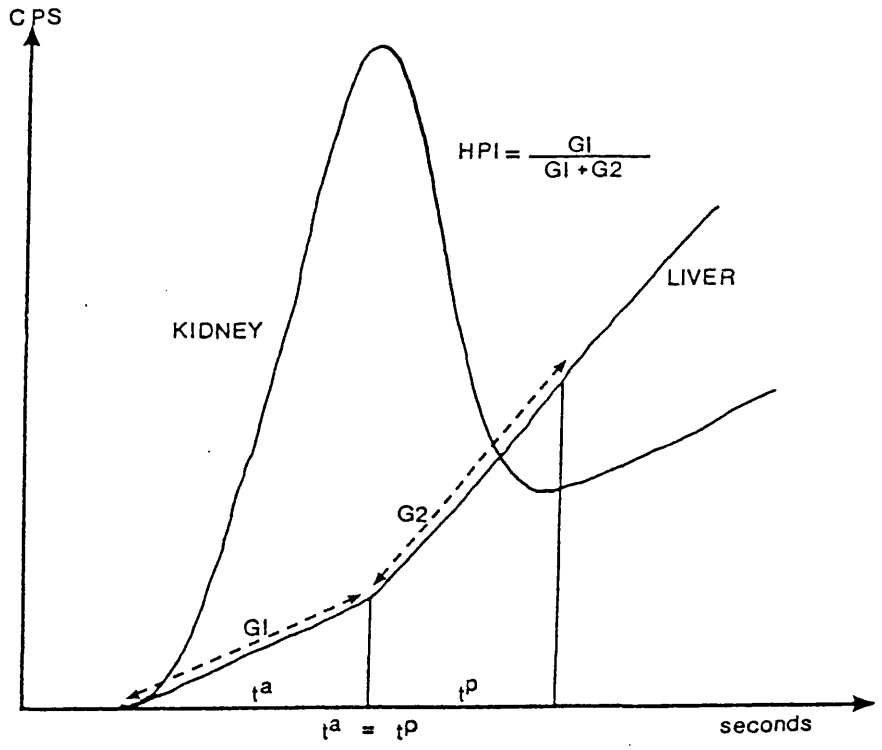


Figure 4: Idealised curves for the derivation of the HPI(Leveson et al,1985)

VALIDATION OF THE MODIFIED TECHNIQUE

The inter- and intraobserver variation were assessed on eighteen consecutive patients presenting with colorectal cancer. Each underwent dynamic scintigraphy using the modified high administered activity technique described above. To assess intra-observer variation, each study was reprocessed four times,creating four sets of regions of interest and time activity curves.Each study was independently reprocessed by a second observer,creating a fifth set of values. The inter-observer variation was calculated by taking the first value of observer 1 and the value of observer 2,assessing both the positive difference and the root mean square difference of the values. In addition the correlation coefficient of the inter-observer values was calculated.

In clinical practice,the difference between the obtained values is often more important than the degree of similarity or "correlation". This may be assessed using the Bland Altman technique. In this if there is no significant difference,the difference between the values divided by the mean will equal zero,or the ratio of the values divided by the mean will equal one. Whether the difference between or the ratio of the values is used,is dependent on whether the magnitude of the difference increases as the value increases(in which case the ratio should be used). Finally,having established the reproducibility of the technique,the clinical correlation was then assessed on 50 consecutive patients with colorectal carcinoma.

COMPARISON OF METHODS OF DETECTING COLORECTAL METASTASES

Patients referred to the University Department of Surgery were studied. All had a presumptive diagnosis of colorectal carcinoma,which in all but 2 cases was subsequently confirmed on biopsy. All patients were investigated for the presence of hepatic metastases,their subsequent clinical management being determined by a combination of their physical fitness and stage of disease.

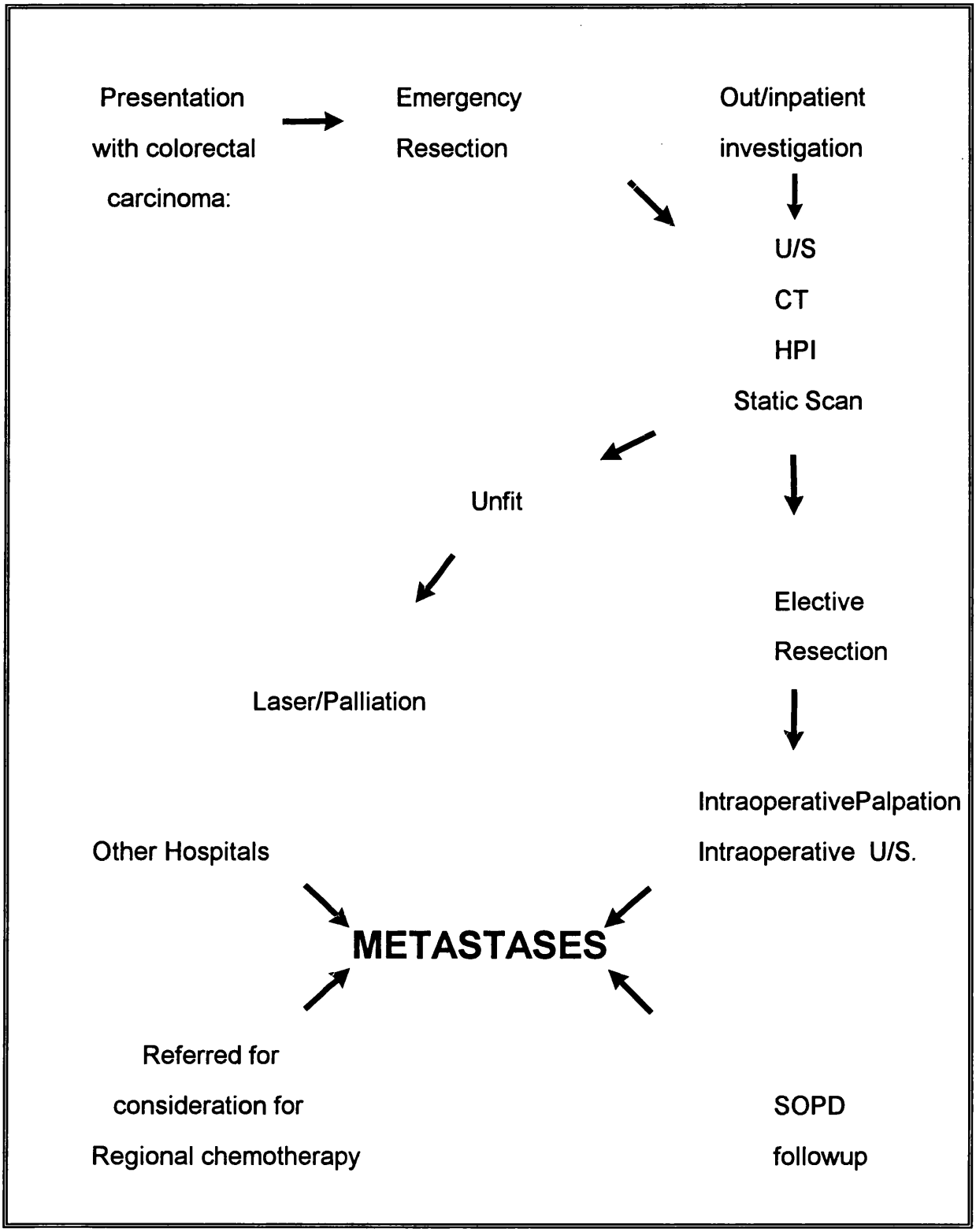
Figure 5 is a flow diagram illustrating the clinical fate of patients included in the study. All were investigated with a minimum of an ultrasound scan,CT scan of the abdomen,static liver scintigraphy and dynamic hepatic scintigraphy(hepatic perfusion index). Latterly,patients also had measurement of blood flow in the hepatic artery and portal vein by duplex Doppler ultrasonography, but his was not available for the whole duration of the study. Those patients that proceeded to elective surgery had the presence or absence of metastases assessed by palpation and in the case of palpably clear livers by intraoperative ultrasound.

Patients were considered to be metastases positive if they were confirmed at laparotomy,biopsy or shown to have disease progression during the followup period.The accuracy with which each of the above investigations identified the presence or absence of hepatic metastases has been prospectively compared,and in particular the clinical application of intraoperative ultrasound has been assessed.

All patients presenting to the University Department of Surgery Glasgow Royal Infirmary with colorectal carcinoma after January 1990, have been included in an imaging programme,to detect the presence or absence of hepatic metastases. Those patients that presented as an emergency were screened in the post-operative period. The remainder presented with symptoms to the emergency department or outpatient clinic,and were investigated prior to elective surgery or laser palliation. In those patients that were unfit for laparotomy or those treated by intraperitoneal chemotherapy via a "tenchkoff" catheter where adequate access is prevented, intraoperative ultrasonography was not performed.

Ultrasonography and contrast enhanced computerised tomography and static hepatic scintigraphy were supervised and reported by consultants in radiology and nuclear medicine. The dynamic scintigraphy was analysed by myself,Mr D Hemingway or G McCurragh. The intraoperative ultrasound was performed by myself or Mr D. Hemingway after an initial training period six months prior to the study to validate the technique.

Figure 5: Clinical course of patients within study



INTRAOPERATIVE ULTRASONOGRAPHY.

The intraoperative ultrasonography was performed using a waterproof T-shaped probe,with a 5 MHz frequency,sterilised by immersion in 2% glutaraldehyde ("cidex") for 10 minutes prior to use. The natural humidity of the liver avoids the need for contact surface gel. Access to the liver parenchyma is limited by the fibrous attachments of the liver to the abdominal wall and diaphragm(falciform ligament,right and left coronary ligaments and the bare area).

The potential advantage intraoperative ultrasound holds over transabdominal ultrasound lies in the requirement for less tissue penetration. Penetration reduces as the frequency increases,and the resolution increases with frequency. Consequently whereas a transabdominal probe would usually be 2 - 3 MHz(15cm penetration), with a minimum resolution of 4-5 mm, a intraoperative probe usually employs a 5MHz probe,with less penetration (approximately 8 cm) but better resolution(2-3mm).

In one patient dense fibrous adhesions prevented adequate access to the left lobe. There is also a "blind zone" 1 - 2 cm from the surface. This can be minimised by altering the frequency,or visualisation via a water cushion if doubt exists. Lesions in this region are usually clinically apparent,and the additional time required to use the cushion unnecessary.

The probe is placed transversely on the anterosuperior aspect of the right lobe to visualise the confluence of the three hepatic veins with the inferior vena cava. These can then be followed to identify the segmental venous drainage. The portal venous radicles are then identified,being distinguishable from the hepatic veins by their echogenic fibrous envelope derived from "Glisson's capsule" These are then followed down to the porta hepatis. The gallbladder and hepatic pedicle

are then studied by turning the probe longitudinally lying anteriorly on the caudate lobe. Segments II and III are then visualised to the left of the falciform ligament. Metastases (Figure 6) are identified by their heterogenous echo pattern, and their segmental location noted. Abnormalities may be recorded on a Sony AX 24 printer,integrated with the ultrasound machine.

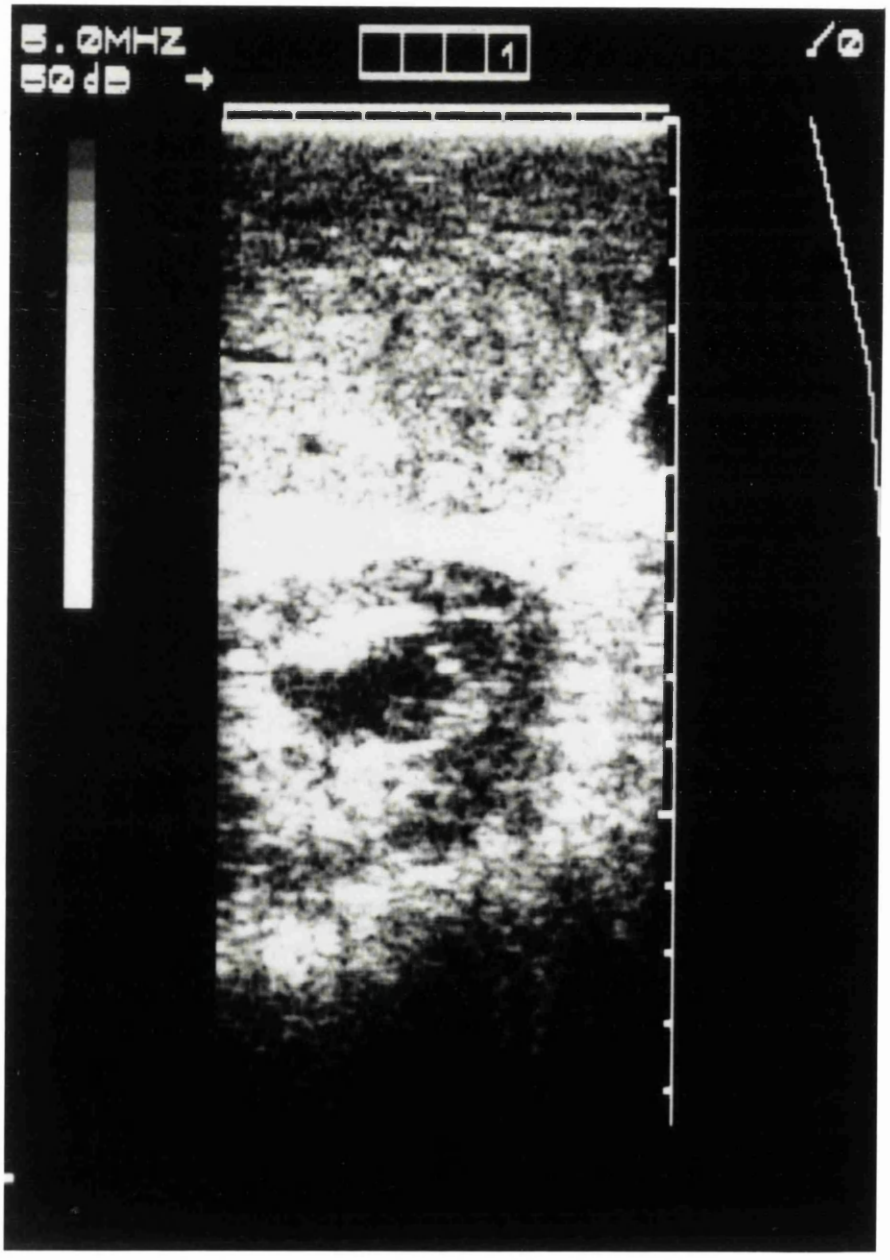


Figure 6: Intraoperative ultrasound of a two metastases lying in segment VII of the liver.

COMPARATIVE STUDY OF METHODS FOR DETECTING COLORECTAL METASTASES.

All six detection modalities were completed in 40 patients. Having completed all investigations,patients were considered positive for metastases if they had histological or post mortem evidence of colorectal metastases, or had retrospective evidence of disease progression on serial followup investigations. The sensitivity(The number of true positives as a percentage of the total with metastases),specificity(the number of true negatives divided by the number that are disease free),and positive (proportion of true cases among those with a positive result) and negative(proportion of true negative cases among those with a negative result) predictive value of each investigation was calculated.

In a further 33 patients one or more investigations was not performed for reasons described earlier,however, the accuracy of the performed investigations was assessed,to determine whether the figures obtained from the comparative study were maintained in this larger cohort of patients.

SECTION II - HAEMODYNAMIC STUDIES

CELL CULTURE TECHNIQUES AND ANIMAL MODELS:

One of the principal objectives of this thesis was to investigate the mechanisms that underlie the changes in the hepatic perfusion index that occur with the development of intrahepatic tumour. The methods available for the assessment of hepatic anatomy and blood flow are either unsuitable for use *in-vivo* (colloidal dyes,casting techniques), provide information on total liver blood flow but do not distinguish between hepatic arterial flow and portal flow(indocyanine green, galactose clearance,BSP),or assess relative portal and hepatic arterial flow but are not quantitative(HPI,DPI).

Similarly,methods for studying drug distribution are limited in clinical research by the availability of tissue for analysis. Consequently despite the difficulties in correlating animal based studies with clinical practice,we chose to employ an animal model of hepatic metastases.

Throughout this series of experiments the following rodent model of colorectal metastases has been employed. The cell line used is the HSN fibrosarcoma,and although established within the University Department of Surgery,was originally gifted by Dr. S Eccles, Institute of Cancer Research,Royal Marsden Hospital. Previous experiments in the department have shown that this cell line grew well in immunocompetent rats, producing discrete tumours two to three weeks following inoculation, and,as in a proportion of human colorectal metastases,these tumours were hypovascular relative to surrounding liver parenchyma,and derived the majority of their blood supply from the hepatic artery(Nott et al,1989,Hemingway et al,1991).

Preparation of the cell suspension for inoculation:

Approximately 10 ml of culture medium (50 mls fetal calf serum,10 ml penicillin/streptomycin solution(Sigma),10 ml Glutamine (200mmol solution),and 500ml Dulbecco's Modified Eagles Medium) was aspirated from a flask containing a confluent monolayer of HSN cells and was discarded. The cells were washed in sterile PBS which was again aspirated and discarded. Ten ml of 0.1% trypsin solution (Sigma) was added and the flask incubated at 37^{o}C. The cells were inspected under phase contrast microscopy intermittently until the cells were seen to have gone into suspension. The trypsin was inactivated by the addition of 5 mls fetal calf serum,the suspension being placed in a universal container. Following centrifugation the supernatant was discarded and the pellet resuspended in fresh medium,the viability being confirmed microscopically by the addition of trypan blue, additional medium adjusting the concentration to 10^6 cells per ml.

Tumour induction:

Male hooded Lister rats weighing approximately 200 - 250g, are anaesthetised by an intraperitoneal injection of sodium pentobarbitone (Sagatal,30 mg/kg). An upper midline laparotomy was performed and the antero-superior surface of the liver exposed. An intrahepatic injection of 10^5 HSN Sarcoma cells suspended in 0.1 ml of fetal calf serum as previously described was given into the right and left lobes of the liver,taking care to avoid spillage.Haemostasis was achieved using pressure applied with cotton buds soaked in ethanol,to avoid peritoneal contamination. The abdomen was closed in two layers using 2.0 dexon sutures. All experiments were performed between 15 and 25 days following inoculation to correspond with the rapid growth phase of the HSN cell line growth curve.

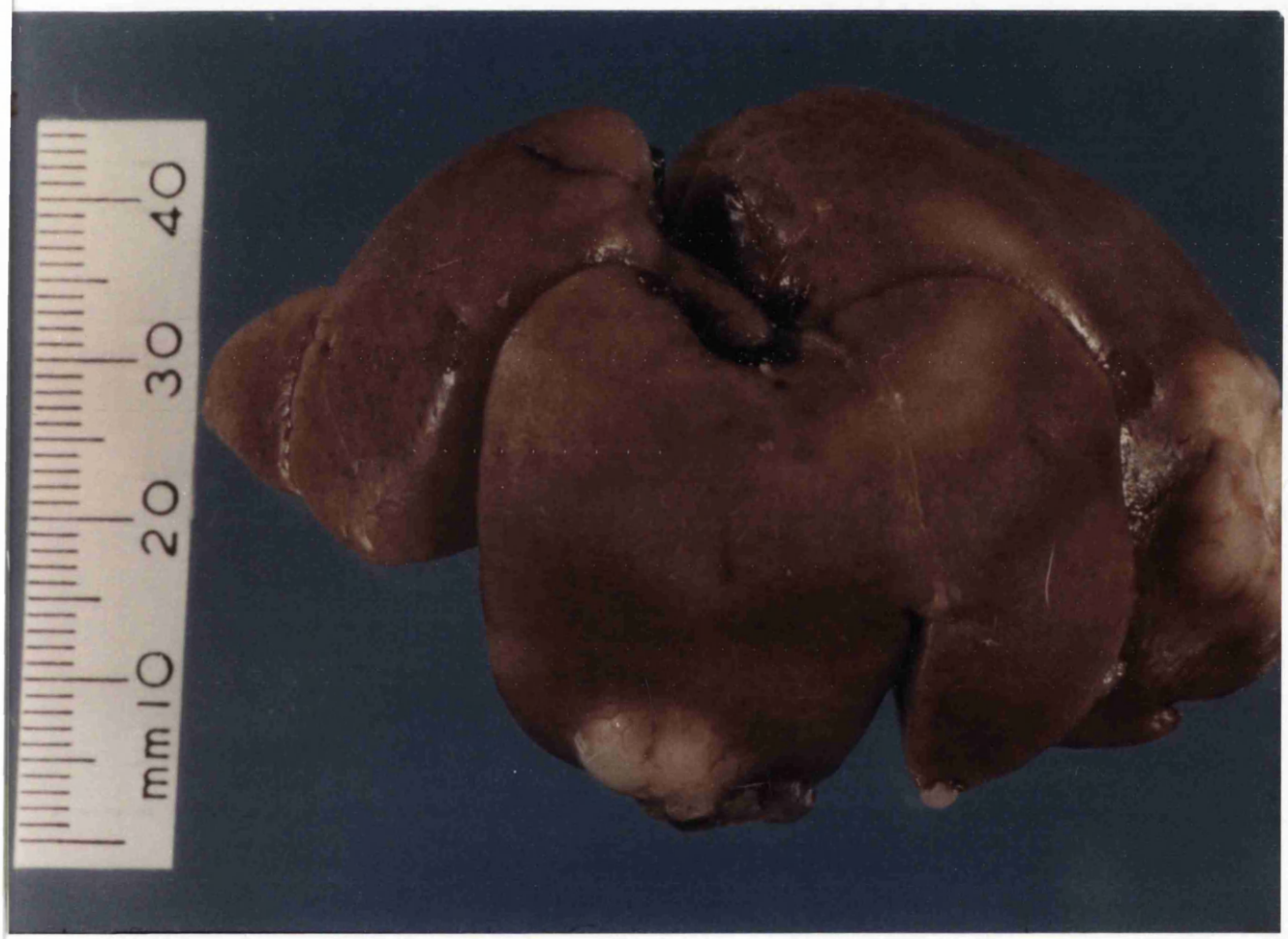


Figure 7: HSN tumour at 20 days.

CROSS-PERFUSION EXPERIMENT

As discussed in the introduction,persistent alterations in splanchnic blood flow are determined by neural and humoral influences. This experiment was designed to investigate the hypothesis that the relative reduction in portal flow was caused by the presence of a circulating vasoactive agent. The blood from a tumour bearing animal may therefore result in similar hepatic haemodynamic changes when perfused through a second,otherwise normal,animal.

Preparation of the experimental animal:

After an overnight fast,a rat weighing approximately 400g,was anaesthetised by an intraperitoneal injection of sodium pentobarbitone (Sagatal 30mg/kg),and a transverse dorsal neck incision performed. The platysma was divided and the strap muscles split in the midline to expose the trachea. A transverse tracheotomy was performed and a 6FG tracheostomy inserted and secured. The plane between the strap muscles and the sternomastoid was developed to expose the carotid artery which was stretched and cannulated with a specially constructed cannula, the tip of which had an external diameter of 1mm(internal diameter 0.85mm).

After 15mm the cannula diameter expanded to 2mm,to minimise the resistance of the extracorporeal circuit. It was connected to a staggered multiport connector which permitted continuous monitoring of the systemic arterial pressure and subsequent extracorporeal autoperfusion of the intestinal segment. The jugular vein was cannulated with a 5FG flexible cannula.

Maintenance of anaesthesia:

The animal was mechanically ventilated with an inspired gas mixture of 50/50 NO/O_2,a stroke volume of 25ml and a respiratory rate of 40/minute determined by blood gas analysis to maintain the pO_2 above 100mmHg and the pCO_2 below 40mmHg. Repeated 0.1 ml boluses of 8.4% $NaHCO_3$ were required at 30 minute intervals to prevent the development of a metabolic acidosis. Further intravenous boluses of sodium pentobarbitone(0.1ml) were given if the depth of anaesthesia showed any signs of lightening. Using an oesophageal temperature probe and heat lamps the animals temperature was maintained above 36^oC.

Preparation of the segment:

The animal was anticoagulated using sodium heparin (200 units/100g) and a midline laparotomy performed. The small bowel was wrapped in cling film and retracted to the right to allow access to the distal colon which was mobilised and divided at the level of the pelvic brim. The colon was then followed proximally to the level of the duodenal-jejunal flexure to identify the left colic vessels which were divided. The terminal ileum was then tied and divided at the level of the terminal arcade,the ileocolic vessels being tied distal to the last vessel to the ileum. The plane between the colon and small bowel mesentery was then developed up to the level of the right colic vessels. The colon was then reflected caudally to allow dissection of the pancreas from the mesocolon. Having achieved this the colic vessels were divided and the colon removed.

The spleen was then retracted caudally and the stomach cephalad,the adventitial tissue between these being divided up to the level of the short gastric vessels, which were tied in continuity before division. There is no surgical plane between the pancreas and stomach, both being fed by vessels of the right

gastroepiploic artery. The gastroepiploic was therefore divided along the inferior border of the stomach to the level of the pylorus.

The lesser omentum was then opened allowing the pylorus to be ligated and divided, the duodenum being retracted laterally. This allows identification of the coeliac artery arising from the aorta. The coeliac artery was followed distally, to where it gives off the hepatic artery which was dissected off the portal vein and divided.

The coeliac artery itself was tied flush with the aorta and then divided. A plane can be identified between that part of the pancreas supplied by the coeliac artery and that supplied by the superior mesenteric artery. By developing this plane the portal vein can be identified, vessels draining the distal pancreas arising to the left of the portal vein and those from the head of the pancreas emerging from the right. These vessels were carefully identified, diathermied and divided. The spleen and pancreas were removed, and the small bowel divided at the level of the duodeno-jejunal flexure to skeletalise the superior mesenteric artery and portal vein.

The superior mesenteric artery was then quickly cannulated,using another tapering cannula connected to the multiport connector,with a terminal internal diameter of 1mm. The arterial half of the extracorporeal circuit was allowed to perfuse for 5 minutes to ensure adequate oxygenation of the segment. The portal vein was then cannulated using a 6 FG flexible cannula,the emerging blood being collected in a measuring reservoir before being recirculated using a rotary pump into the internal jugular vein. (See figure 8)

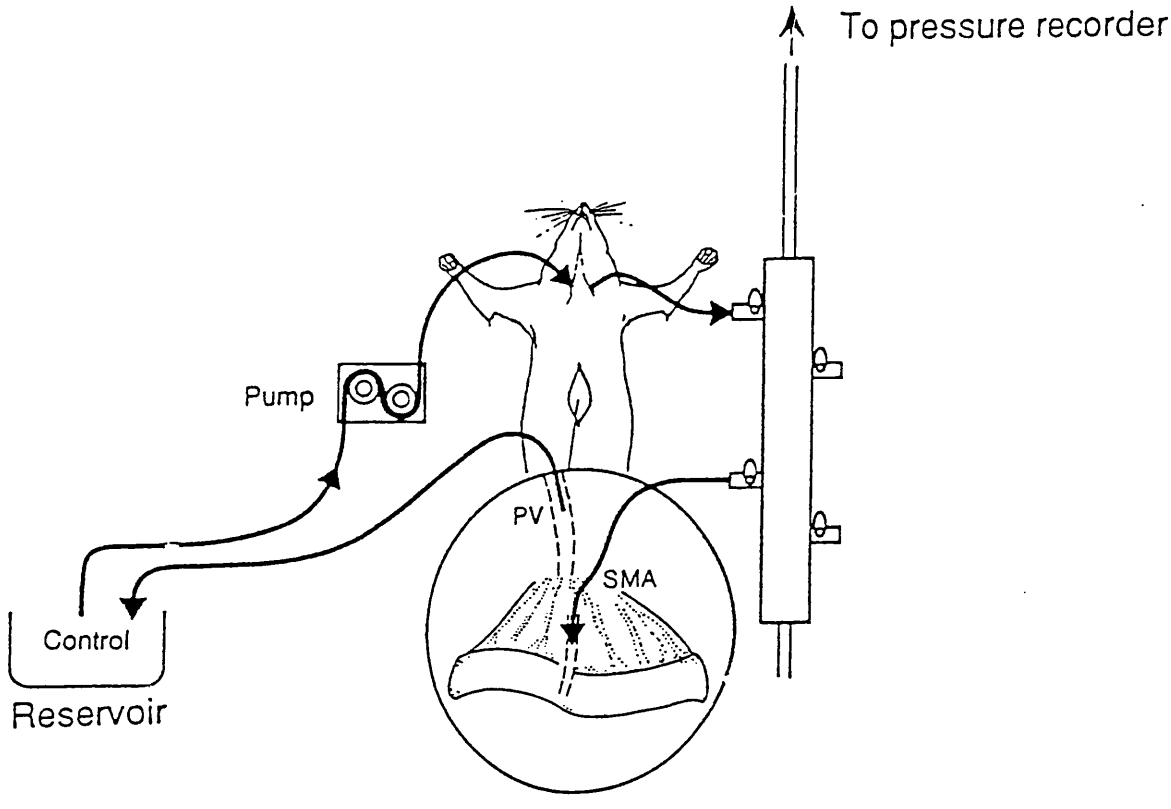


Figure 8: Establishment of an extracorporeal circuit to perfuse the isolated segment.

Preparation of the donor animals

Two donor animals,syngeneic Hooded Lister rats, were anaesthetised using an intraperitoneal injection of sodium pentobarbitone(Sagatal 30mg/kg). One of these had undergone intrahepatic tumour induction three weeks earlier as described previously, the other was a control. In each animal a tracheostomy was performed and the carotid artery cannulated using a 2-1mm tapering cannula. A 5FG cannula was inserted into the right jugular vein for the return of blood from the reservoir, the animals being heparinised with an intravenous injection of sodium heparin (200units/100g).

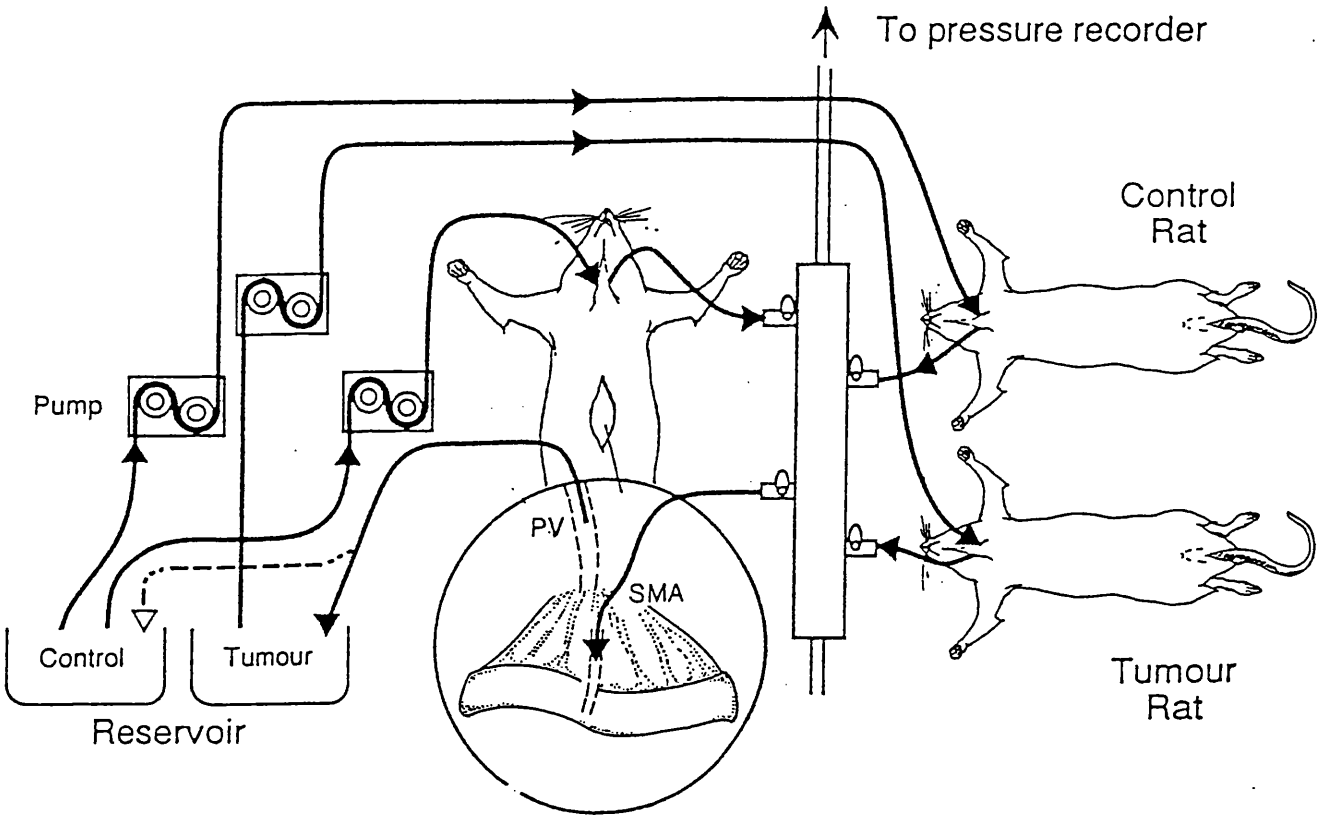


Figure 9 : Connection of the donor animals to the circuit.

Establishment of the cross perfusion circuit.

The cross perfusion circuit is summarised in figure 10. Using a staggered multiport vascular access "traffic light" the carotid cannulae were connected prior to establishment of the cross perfusion to allow rapid crossover limiting interval ischaemia. The initial blood emerging from the segment following crossover was discarded to minimise venous mixing.

Two syngeneic rats,one tumour bearing,one control, were anaesthetised and heparinised as previously described. A midline laparotomy was performed and the aorta cannulated. The animals were exsanguinated providing fresh,heparinised blood to prime the reservoirs and the venous return cannulae.

The isolated small bowel segment was then perfused by one of the "donor" rats followed by the other the order being determined by a computerised random number sequence. The blood flow through the segment was measured over three 30 second periods,each 90 seconds apart,collecting the blood emerging from the portal venous catheter in a measuring chamber before returning the blood to the reservoir. A five minute equilibration period was allowed to elapse before measuring the flow attributable to each animal.The flow achieved at each time point was combined with the simultaneous mean arterial pressure to calculate the mean vascular resistance of the segment using the formula:

$$\text{RESISTANCE} \; (\text{mmHg.ml}^{-1}.\text{min}^{-1}) = \frac{\text{PRESSURE}}{\text{FLOW}}$$

Further cross perfusions were performed on each segment but due to the inevitable minor mixing of tumour and control blood only the first measurements for each animal were used for statistical analysis. The significance of the observed differences were assessed using the Mann-Whitney U test, and the Wilcoxon rank sum test for paired data.

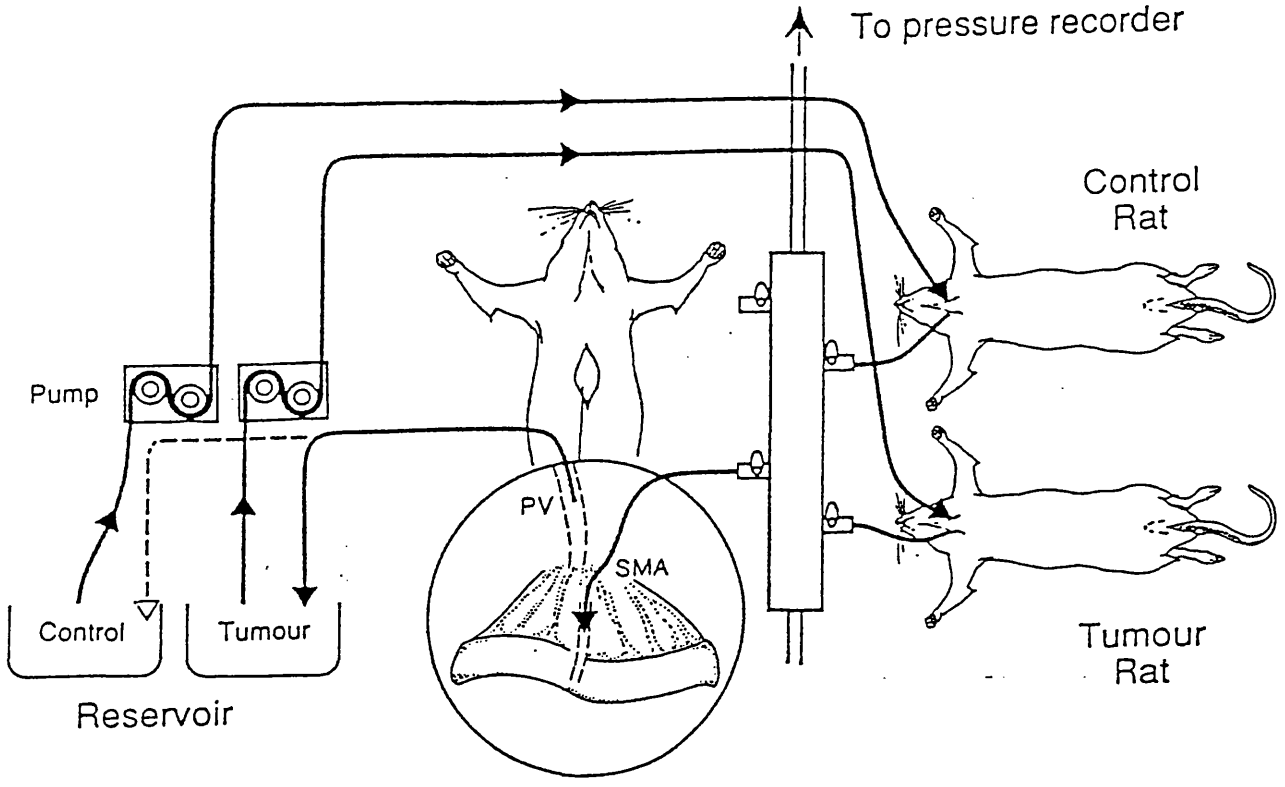


Figure 10: Experimental animal excluded from the cross perfusion circuit

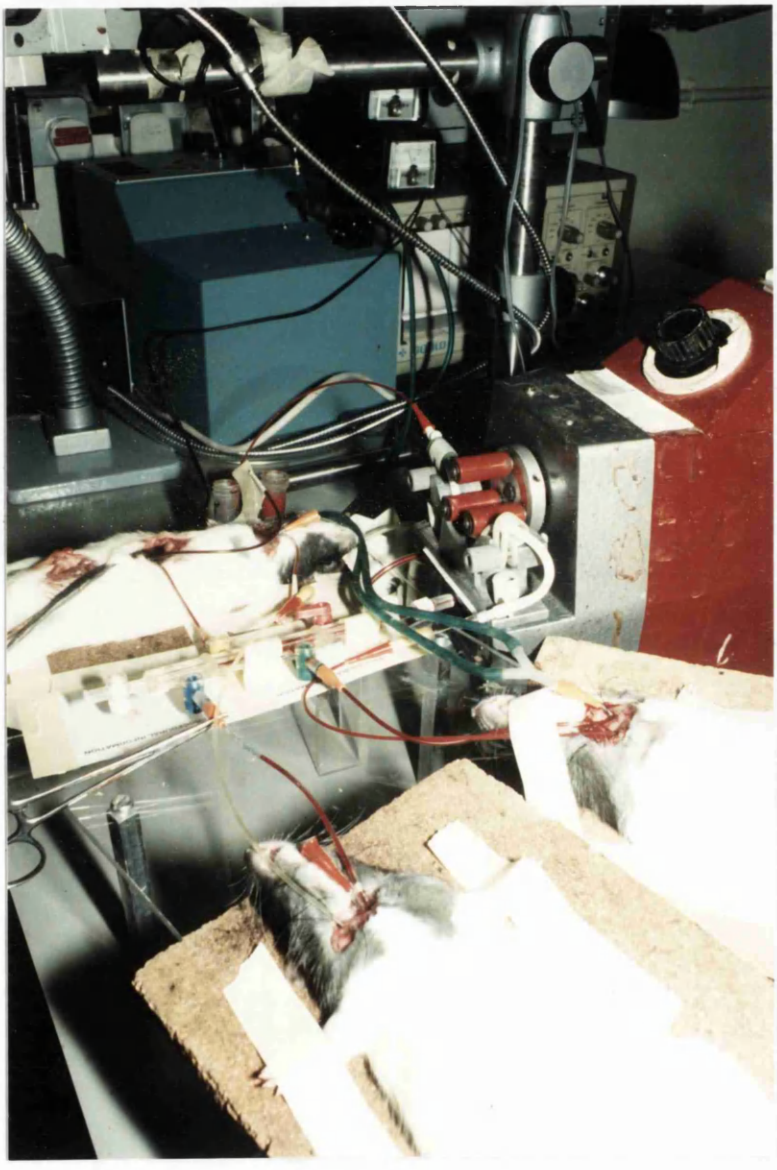


Figure 11 : Photograph of cross perfusion experiment.

REFERENCE MICROSPHERE EXPERIMENTS

If the mechanism underlying these haemodynamic changes is as the result of a reaction between the tumour and host,this raises the question as to why the HPI alters only with the development of secondary tumour and is not effected by the primary tumour. This experiment was designed to assess the effect of extrahepatic tumour on liver blood flow.

Hooded Lister rats weighing 200-250 g were used throughout the experiment. Three groups of animal were studied:

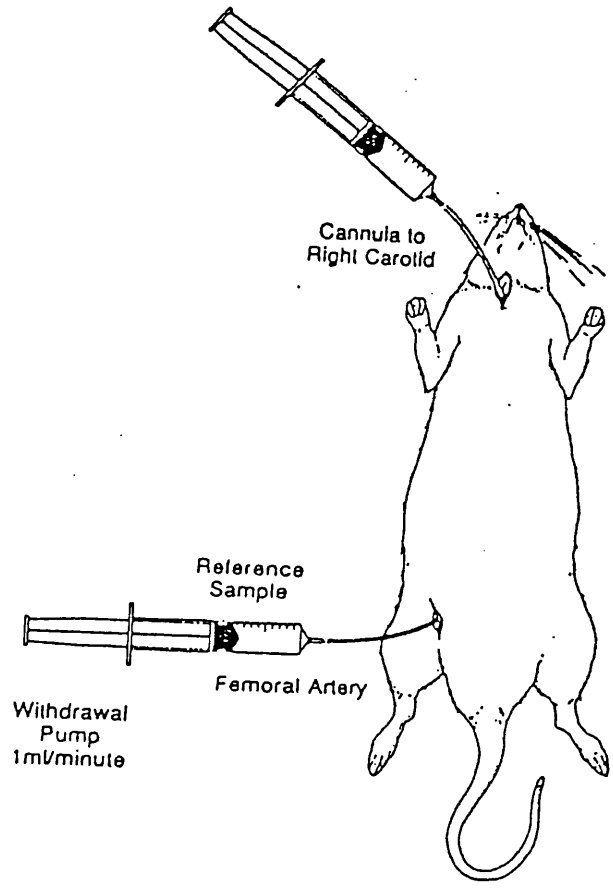
(1) Control animals that had undergone a laparotomy and intrahepatic injection of dead tumour cells.

(2) animals that had undergone intrahepatic tumour induction as described previously,and

(3) animals that had subcutaneous tumour induced by a subcutaneous injection of 10^6 HSN sarcoma cells, producing 10-15mm tumours three weeks after inoculation.

The animals were anaesthetised by an intraperitoneal injection of sodium pentobarbitone(Sagatal 30 mg/kg)and a left groin incision performed. The left common femoral artery was isolated and ligated distally before being stretched and cannulated with a 1mm internal diameter cannula. The cannula was flushed with heparinised saline, and connected to a 2ml syringe in a withdrawal pump calibrated at 1ml/min.

Figure 12 : The Reference Microsphere Sample Technique



$$\text{Hepatic arterial flow} = \frac{\text{counts in liver}}{\text{counts in reference}} \times \text{reference sample withdrawal rate.}$$

$$\text{Splanchnic flow} = \frac{\text{counts in splanchnic organs}}{\text{counts in reference}} \times \text{reference sample withdrawal rate.}$$

$$\text{Hepatic perfusion Index(HPI)} = \frac{\text{Hepatic arterial flow}}{\text{Hepatic + splanchnic flow}}$$

A transverse neck incision was then performed and the platysma divided allowing access to the strap muscles which were split in the midline. A 6 FG tracheostomy tube was inserted to ensure a patent airway,the animal breathing spontaneously using an enriched-oxygen inspired air mixture. The right internal carotid artery was identified lying in the groove between the strap muscles and the sternomastoid. This was ligated distally and cannulated with a 0.75mm internal diameter cannula. This, when connected to a pressure monitor,allowed placement of the catheter into the left ventricle,its position being confirmed by the establishment of a ventricular type pressure tracing.

The 15 μm diameter resin microspheres (Nentrac,Dupont Ltd) labelled with ^{153}Gd,in a concentration of approximately 5 x 10^5 microspheres/ml, were suspended in saline and agitated for two minutes. Having ensured that the femoral cannula was flowing,a 0.2 ml intraventricular bolus injection of the microsphere suspension was performed,blood being withdrawn from the femoral cannula over the subsequent 60 second period.

The animal was humanely killed and the organs(Liver,tumour,kidneys, spleen,heart,lungs,stomach,pancreas,small bowel and colon) weighed, divided and placed in a well gamma counter for analysis. Counts per organ were measured and the blood flow for each calculated from the amount in the known reference sample(1ml/min). Animals were excluded from analysis if the flow to the left and right kidney differed by over 15%, as this was taken to reflect incomplete mixing of microspheres with arterial blood.

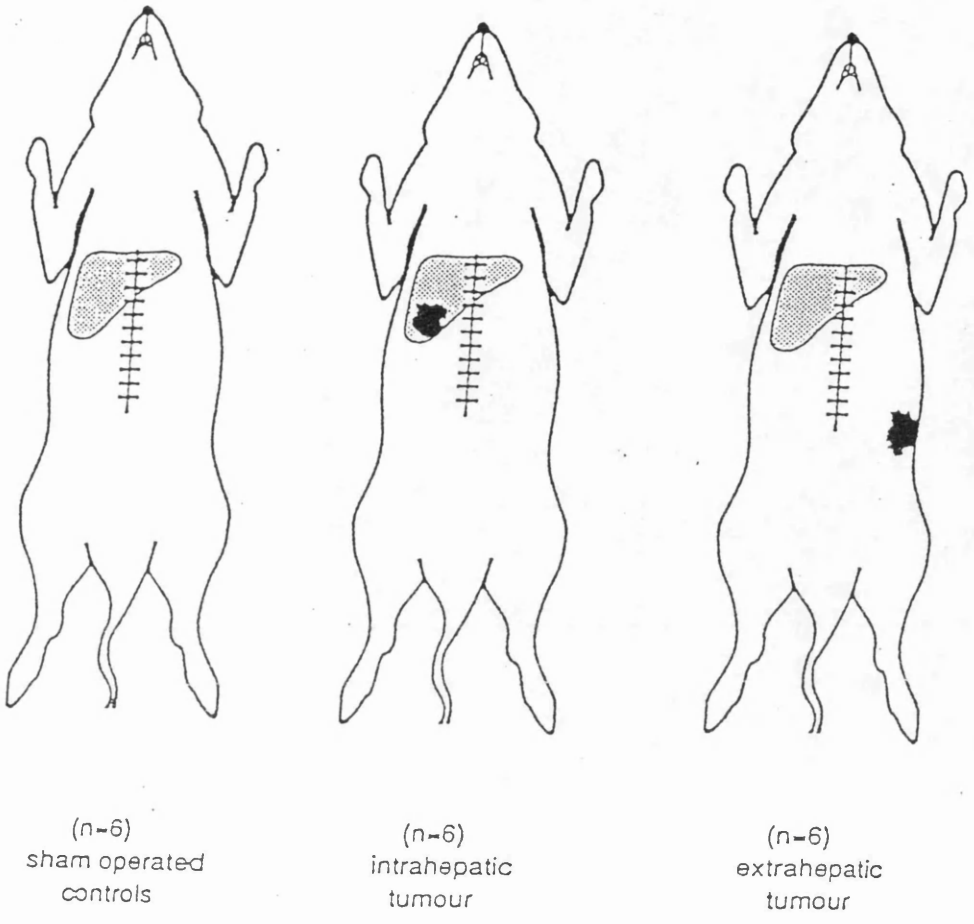


Figure 13: Reference microsphere study design:

The radioactivity in the liver reflected only the hepatic arterial component of blood flow. The portal venous inflow was calculated as the sum of flows to the stomach,spleen,pancreas,small bowel and colon. These data allowed estimation of the hepatic perfusion index(hepatic arterial inflow/ hepatic arterial + portal inflow) for each animal. The flow per gram of tumour tissue relative to the normal liver parenchyma was also calculated.

The significance of the observed differences were assessed using the Kruskal-Wallis analysis of variance,Mann-Whitney U test, and the Wilcoxon signed ranks test as appropriate.

SECTION III - DRUG DELIVERY/TARGETING

Hepatic targeting of chemotherapeutic agents aims to improve delivery of drug to tumour and at the same time reduce side effects by reducing systemic exposure. All experiments used the HSN model of colorectal metastases as previously described.This series of experiments involved the assessment of methods of either first order localisation,in which I have evaluated the possibility of targeting an intravenous bolus injection towards the liver by exploiting the affinity of galactose residues or porphyrins for hepatocytes or second order targeting,where I have investigated how physical microembolisation and vasoactive agents may be combined to improve regional delivery of drugs.

PART 1 - FIRST ORDER TARGETING:

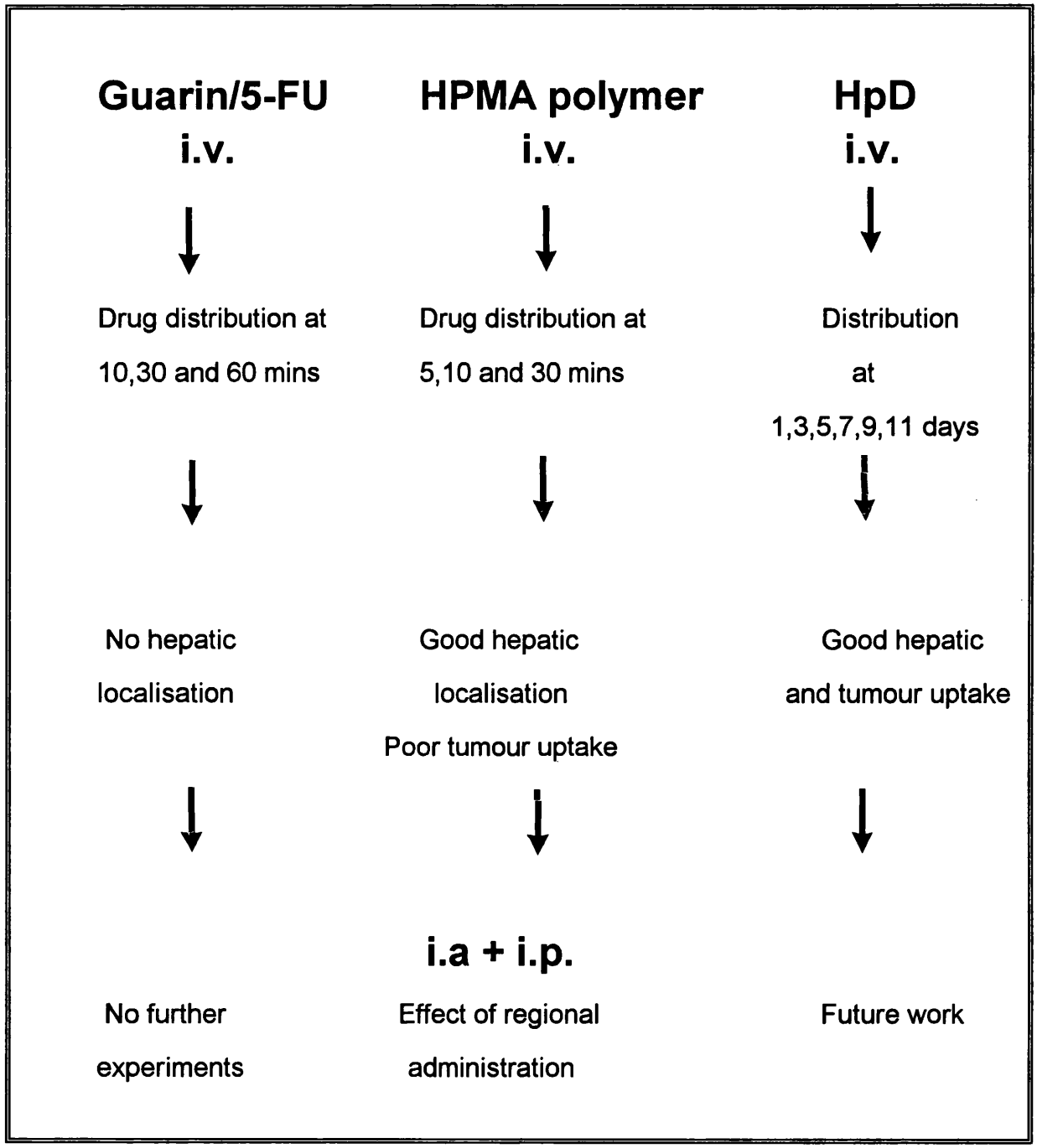
Two potentially useful polymeric compounds conjugated to galactose have been assessed,onto which chemotherapeutic agents could be incorporated,should targeting to either liver or tumour be demonstrated. One polymer used a guarin/polysaccaride backbone the other a hydroxymethacrylamide backbone. Both were radiolabelled allowing systemic distribution to be evaluated following administration. If hepatic targeting of a polymer was demonstrated the effect of regional administration was assessed.

Porphyrins naturally accumulate in tumour and hepatic tissue. In a study by Bugelski,the initial distribution of haematoporphyrin derivative(HpD) matched that of radiolabelled albumen before being cleared from most normal tissue after 6 hours,whereas the levels in tumour tissue remained stable for several days(Bugelski et al,1981). Uptake into hepatocytes was minimal, but accumulated in the Kupffer cells,and other cells of the reticulo-endothelial system.

Correct timing of sampling therefore produces positive tumour to normal tissue concentration ratios for cutaneous tumours,however the ratio attainable with intrahepatic tumour is unknown. The distribution of radiolabelled haematoporphyrin derivative was assessed following intravenous administration into animals inoculated with intrahepatic HSN tumour deposits,with particular reference to variations in the relative tumour and liver tissue concentrations with time following injection.

The significance of the observed differences were assessed using the Kruskal-Wallis analysis of variance,Mann-Whitney U test, and the Wilcoxon signed ranks test as appropriate.

Flowchart summarising the first order targeting experimental plan:



5-FLUOROURACIL/GUARIC ACID POLYMER

Labelling of the polymer:

The 5-flourouracilacetic acid(5-FUAA)/guarin conjugate was developed in the Institute of Medical and Chemical Bioengineering, University of Liverpool. The polymer is a linear manose chain with secondary galactose residues,the 5FU being covalently bonded via the primary carbon of the acetyl linkage, using radiolabelled ^{14}C-5FU obtained from Amersham International ,which was acetylated prior to conjugation. The activity of the 5-FUAA used was $3.48x10^8$ cpm/g;the activity of the resulting conjugate was 9.6×10^6 cpm/g,giving a load of 2.76% in the polymer.

The polymer was prepared as a vacumn dried powder having been purified twice in methanol. This was only slowly soluble in water due to its high molecular weight,initially swelling to give a gel,subsequently becoming more fluid with gentle agitation in a water bath at 37^oC over a 24 hour period to leave a colourless solution.

A recovery experiment was performed to ensure that the volume of tissue in each sample did not effect the sample counts(Appendix 5a). This was performed using free ^{14}C-5-FU acetic acid. The liver from a recently sacrificed animal was divided into cubes of varying sizes(100mg - 600mg approx). Ten microlitres of the 5-FUAA was then injected into the centre of each cube,which was then placed in a scintillation vial,solubilised using 2ml of "soluene" and decolourised with 30% H_2O_2 Having waited a minimum of 6 hours to allow any chemofluorescence to decay, 10ml of "Optiphase" scintillant fluid was added,and the vials counted in a beta scintillation counter,each sample being counted over a ten minute period. Quench correction was performed using the external standard channels ratio technique. The counter was regularly calibrated using a set of commercial standards to generate the quench correction curve.

Animal experiment:

This experiment investigated the differences in organ distribution between free drug(5-FUAA) and the galactose polymer conjugate. Tumour bearing animals were anaesthetised using intra-peritoneal "Sagatal" and a transverse neck incision performed. The trachea was mobilised and a tracheostomy inserted and secured. The internal carotid artery was ligated and cannulated with a 3FG cannula for blood sampling. The right jugular vein was cannulated and each animal then given an 250 μl intravenous bolus injection of either free 5FU acetate or the conjugate. Blood samples were obtained every 2,5 or 10 minutes respectively by collecting dropped blood in a Epindorph vial. Animals were killed after either 10 minutes,30 minutes or 60 minutes.

Thirty microlitre samples of the dropped blood were obtained using a Gilson pipette,new tips being used for each sample to avoid contamination. These were dropped onto a filterpaper disc, to which was added 0.4 ml of 30% H_2O_2 to bleach the sample. Frothing was avoided by adding the peroxide dropwise. The blood pharmacokinetic profile following intravenous injection was assessed by plotting the venous blood concentration(expressed as the percentage of the injected dose per ml) against time and the log concentration against time. In addition, any difference in potential total drug exposure following administration of each compound was assessed by comparing the ratio of the plasma AUC's(area under the time concentration curve for blood);the larger the ratio,the greater the relative total drug exposure .

The liver,tumour, kidneys,heart ,lung and spleen were dissected, subdivided into 200-300mg segments. These were placed in scintillation vials to which was added 2ml of "soluene". The vials were placed in a agitating waterbath for 24 hours to solublise the tissue before adding 1 ml of H_2O_2. All of the samples were left overnight to allow any chemoflourescence to settle before adding 10 of "Optiphase" scintillant fluid to each vial. The samples were counted over a ten minute period,to ensure a minimum of 200 counts per sample.

A 25µl reference sample was taken at the same time as the bolus injection,to which was added "soluene",H_2O_2,and "Optiphase" scintillation fluid prior to counting. The counts were summated for each organ and expressed as a percentage injected dose per gram of tissue. Because the guarin conjugate showed little tendency to accumulate in the liver or tumour no regional administration experiments were performed.

N-(2-HYDROXYPROPYL)METHACRYLAMIDE POLYMER

Labelling of the polymer:

The polymer was provided unlabelled from Professor Ruth Duncan,Keele University. A 2mg sample of the polymer was placed in a sealed vial to which was added 1 ml of phosphate buffered saline,iodobeads(Pierce Chemical Company), and 300 μCi of Na ^{125}I. After incubation for 20 minutes the 1ml solution was placed in a "Centricom 10" centrifuge tube and centrifuged for 30 minutes at 3000 rpm. The supernatant was discarded and the sample washed from the filter membrane using sterile water with reversed centrifugation at 300 rpm for 10 minutes. A drop of the polymer containing fluid was placed on thin layer chromatography paper, and using 85% methanol as a mobile phase separation of the bound and unbound ^{125}I was performed. Activity of the source and solvent front was determined in a well gamma counter,allowing a percentage labelling index to be calculated. The centrifugation was repeated until a labelling index in excess of 90% was achieved(Appendix 5b).

Animal experiments:

The aim of these experiments was to again assess the distribution of the polymer following intravenous administration, and then assess whether the targeting of polymer to liver or tumour could be further improved by regional administration. As in the other distribution experiments the HSN model of colorectal metastases was used.

The polymer was administered in three ways. The distribution following an intravenous bolus injection was assessed to determine a baseline to which the distribution following either intraarterial or intravenous administration could be compared. From pilot studies performed in mice in Keele it was known that the

uptake in the liver was maximal in the first 10 minutes following injection. Consequently animals were sacrificed at 5 minutes,10 minutes and 30 minutes following injection.

Each animal was anaesthetised using an intraperitoneal injection of "Sagatal" and a transverse neck incision performed. The jugular vein and internal carotid artery were cannulated and a tracheostomy performed as previously described. Systemic arterial blood pressure was monitored via the carotid cannula. Through a left groin incision the femoral artery was cannulated for blood sampling. Those animals in the intraarterial and intraportal groups had either the gastroduodenal artery or a distal mesenteric vessel cannulated respectively.

The labelled polymer was administered by a 100 μl bolus injection over 20 seconds.Drop blood samples were collected in Epindorph vials at set intervals prior to sacrifice. One hundred microlitre samples of the dropped blood were obtained using a Gilson pipette,new tips being used for each sample to avoid contamination. Each blood sample was made up to 1 ml using normal saline prior to counting in a well gamma counter. The blood pharmacokinetic profile following intravenous injection was assessed by plotting the venous blood concentration(expressed as the percentage of the injected dose per ml) against time and the log concentration against time. As in the 5-FU distribution studies any regional advantage provided by the different modes of administration was assessed by calculating the median AUC(area under the concentration curve) for each group of animals,and calculating a ratio between intravenous and both intraarterial and intraportal administration..

After the animals were killed, the liver, tumour,kidneys ,spleen,heart and lungs were dissected from the carcass. The carcass and each organ was weighed before being placed 1M NaOH. This was then heated to 85^{o}C dissolving all solid tissue. The solvent volume was measured and five 1ml samples placed in vials allowing calculation of the counts per organ, the results being expressed as a % recovered dose per organ, and % recovered dose per gram of tissue.

^{14}C-HAEMATOPORPHYRIN DERIVATIVE DISTRIBUTION STUDIES:

Labelling of the HpD:

The haematoporphyrin was obtained from Professor S.B. Brown of Leeds Radioporphyrins. This ^{14}C-polyhaematoporphyrin is equivalent in composition to the clinically used photosensitiser,"Photofrin II". Because of the complexity of the labelling process,the radiolabelled HpD is extremely expensive limiting the number of possible experiments.The drug is supplied as a freeze dried powder,which is reconstituted in saline. The dose of Hpd was 5mg/kg(as in the clinical application),each animal receiving 1 mg Hpd (labelled with 0.5 μCi ^{14}C to provide sufficient counts to be available despite excretion). The freeze dried powder was then reconstituted in 0.9% saline for injection.

Animal experiments:

The aim of these experiments was to assess the distribution of the HpD following intravenous administration,and evaluate how the distribution and more particularly the tumour to normal liver ratios varied with the duration following injection. As in the other distribution experiments the HSN model of colorectal metastases was used. Fifteen days following the induction of intrahepatic tumour,each animal was anaesthetised using an intraperitoneal injection of "Sagatal" and a transverse neck incision performed. The jugular vein was exposed and the Hpd given by slow intravenous injection.

The animals were allowed to recover and 2 animals subsequently killed at 1,3,5,7,9,and 11 days following injection. The liver, tumour,kidneys, spleen, heart and lungs were dissected from the carcass. In addition a section of the pelt was removed and weighed. The carcass and each organ was weighed before being

placed in 1M NaOH. This was then heated to 85°C dissolving all solid tissue. The solvent volume was measured and five 0.5 ml samples placed in scintillation vials. To each vial 1 ml of 30% H_2O_2 was added to bleach the solublised tissue. Having left the samples over night to allow any chemoflourescence to settle, 10ml of "Optiphase" scintillant fluid was added. Each sample was counted over a ten minute period,to ensure a minimum of 200 counts per sample.Quench correction was performed using the external standard channels ratio technique. The counter was regularly calibrated using a set of commercial standards to generate the quench correction curve.

The mean counts measured for each organ was corrected for the solvent volume allowing the total counts per organ to be calculated, the results being expressed as a % recovered dose and % recovered dose per gram of tissue.

PART 2 - SECOND ORDER TARGETING

In the presence of overt hepatic deposits drug delivery may be enhanced by regional delivery into the hepatic artery. Previous work from within the department had demonstrated that delivery could be further enhanced by manipulation of blood flow by either vasoactive agents (Hemingway et al,1991) or physical embolisation using degradable starch microspheres(Cooke and Chang,1990). I have investigated the theory that these methods could be combined to further improve drug delivery to tumour.

Liver tumours were induced in male hooded lister rats as previously described. Animals 20 days following tumour induction were anaesthetised by an intraperitoneal injection of sodium pentobarbitone(Sagatal 30 $mg.kg^{-1}$) and the gastroduodenal artery cannulated. Care was taken to ensure that the tip of the cannula lay at the junction of the coeliac and hepatic arteries. A trial injection of saline ensured that the injectate flowed along the hepatic artery and not in a retrograde manner down the coeliac artery. The right common carotid artery was then cannulated for continuous measurement of the systemic arterial blood pressure,via a strain gauge transducer driving a pen recorder(Gould Medical, Lutterworth,UK.).

The dose of Angiotensin II(50μl of angiotensin II $5\mu g.ml^{-1}$ obtained from Ciba-Geigy (i.e 25μl bolus)) and DSM (20μl of DSM solution, $100mg.ml^{-1}$ (i.e 2mg bolus)) were chosen allow comparison with previous experiments(Hemingway et al,1991;Cooke and Chang,1990). Distribution studies were performed using ^{99m}Tc-labelled methylene diphosphonate (MDP),a marker of similar molecular weight as Adriamycin.

Pilot study:

A pilot study, three animal per group, was performed to ascertain the most effective mode of administration of the targeting agents. The following schedules were compared using:

(1) DSM followed by angiotensin II and MDP; or
(2) Angiotensin II followed by DSM and MDP; or
(3) DSM,Angiotensin II,and MDP given simultaneously.

The results of this study suggested that the most effective combination was achieved by the slow bolus injection of Angiotensin II, followed one minute later by degradable starch microspheres and 30μl of ^{99m}Tc-labelled MDP (100 MBq/ml) over 30 seconds. This schedule was therefore used in the main comparative study.(See figures 48 and 49)

Comparative study:

Three experimental groups receiving regional delivery of ^{99m}Tc-labelled methylene diphosphonate(MDP),100 MBq.ml^{-1},were compared. Control animals (two groups of nine) received an intra-arterial injection of 30μl of MDP in physiological saline over 30 seconds. Two groups of animals (n=12) were given a 20μl intra-arterial injection of degradable starch microspheres (100mg.ml^{-1}) mixed with 30μl of MDP as described by Cooke (Cooke and Chang,1990).

In two further groups of experimental animals(12 per group) 50μl of angiotensin II (5μg.ml^{-1}),was injected into the gastroduodenal artery over 30 seconds(Hemingway et al,1990),Systemic arterial blood pressure was monitored and one animal was rejected from further analysis when a rise in mean arterial

blood pressure did not occur. One minute later, chosen as the time point at which the pressor effect is maximal, an injection of degradable starch microspheres(2mg) with 30μl of ^{99m}Tc MDP was performed.

Animals were sacrificed 1 and 90 minutes following injection. The liver was removed and the tumour tissue carefully dissected from the surrounding normal liver tissue. The liver was divided into lobes to ensure there was no intrahepatic distribution variation. The tissue was divided,weighed and placed in vials for immediate counting in a well gamma counter. A reference sample of the ^{99m}Tc MDP was taken at the time of the hepatic injection and counted prior to the samples. Counts were corrected for decay of the ^{99m}Tc.

The results were expressed both as a percentage of the injected dose per gram of tissue,and as a ratio of the relative counts detected in tumour and normal liver tissue. The significance of the observed differences were assessed using the Kruskal-Wallis analysis of variance, Mann-Whitney U test, and the Wilcoxon signed ranks test as appropriate.

CHAPTER 3

RESULTS

As in the previous chapters,this is divided into three sections. Firstly I will describe the results of the clinical studies on the reproducibility of the modified (high administered activity) hepatic perfusion index technique,a comparative study of the sensitivity of modern imaging techniques. I will then describe the results to the investigations of the tumour model itself ,and then the results of the animal experiments investigating why the HPI changes with the development of liver tumour.

Lastly I will describe the results of the animal experiments of drug targeting,investigating firstly,biochemical carriers for organ specific targeting given systemically or regionally,and secondly the combination of physical embolisation and a vasoactive agent to improve the retention of a regionally delivered marker within tumour deposits.

SECTION 1

CLINICAL STUDIES

(1)Reproducibility of the Hepatic Perfusion Index:

Eighteen consecutive patients with colorectal carcinoma were suitable for analysis,and both observers were able to able to generate regions of interest and generate time activity curves for all patients. Ten patients were male and eight female with a median age of 72(range 49-82 yrs).The values obtained are detailed in appendix 1.

Six patients had pathological or radiological evidence of hepatic metastases. The median HPI value obtained in these patients was 0.56(0.38-0.91) compared to 0.33(0.15-0.57) in the other 12 patients without evidence of metastases. Six of these had a value greater than normal(0.37(Parkin et al,1983)),although four of these were within 5% of a normal value. Clinical followup is ongoing.

For a single observer,the median range of reprocessings was 0.035 and the maximum range was 0.11. The median difference between two observers was 0.04(range 0.01-0.11),and the root mean square difference in the HPI

measurements was 0.028,which is 7.5% of the overall median HPI value for observer one. The correlation coefficient between the two observers was 0.98($p<0.001$). The gradient of the regression line was 0.92 with an intercept at 0.07(see figure 14),indicating that observer two was obtaining values slightly greater than observer one.

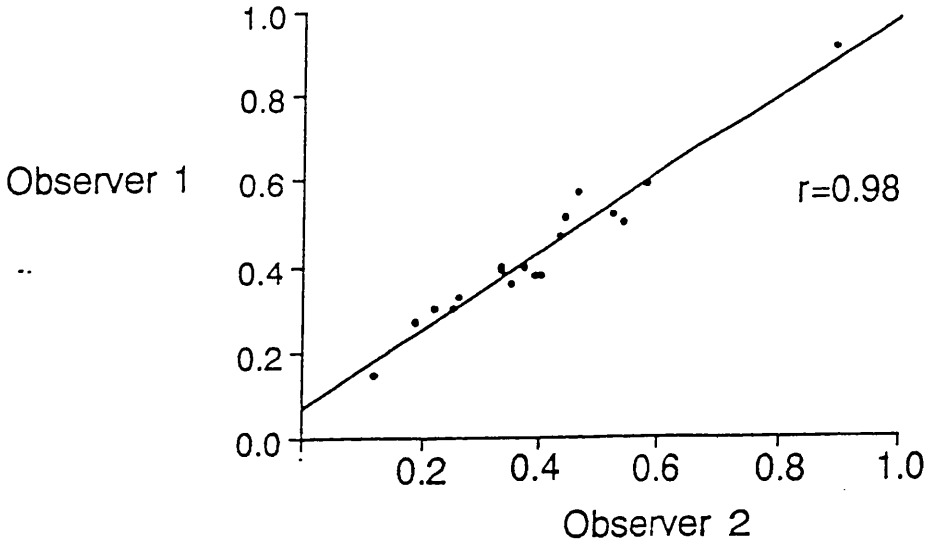


Figure 14 : Correlation between value obtained for the HPI by two observers.

As can be seen from the Bland Altman plot(figure 15),the difference between the values does not increase proportionately with the magnitude of the value. In this case,therefore,the difference between the values,rather than the ratio should be used to determine to what extent the values differ. For two observers, the mean difference between observations was 0.037, with a Standard Error of 0.0096 and Standard Deviation of 0.041. The plot also illustrates,that observer two consistently obtained values greater than observer one.

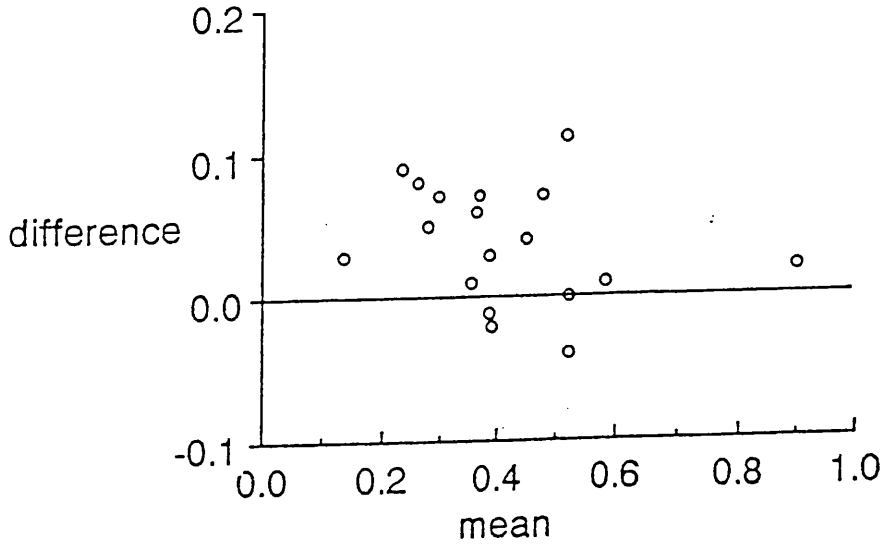


Figure 15 : Bland Altman plot assessing differences between observed values.

Clinical correlation:

The clinical correlation of the modified technique was then assessed on the next fifty consecutive patients presenting with colorectal carcinoma. Twenty-seven were male and 23 female with a median age of 66 yrs(range 48 - 79). Twenty-nine patients had radiological or pathological evidence of metastases. This high percentage(58%) reflects the secondary referral of patients with metastases to the University Department for hepatic chemotherapy or palliative laser treatment. Twenty-one patients were considered disease free. Of the patients with metastases 97% had an abnormal HPI,whereas only 46% considered disease free had a normal value. The prognostic value of an abnormal HPI in patients without evidence of metastases is currently under evaluation.

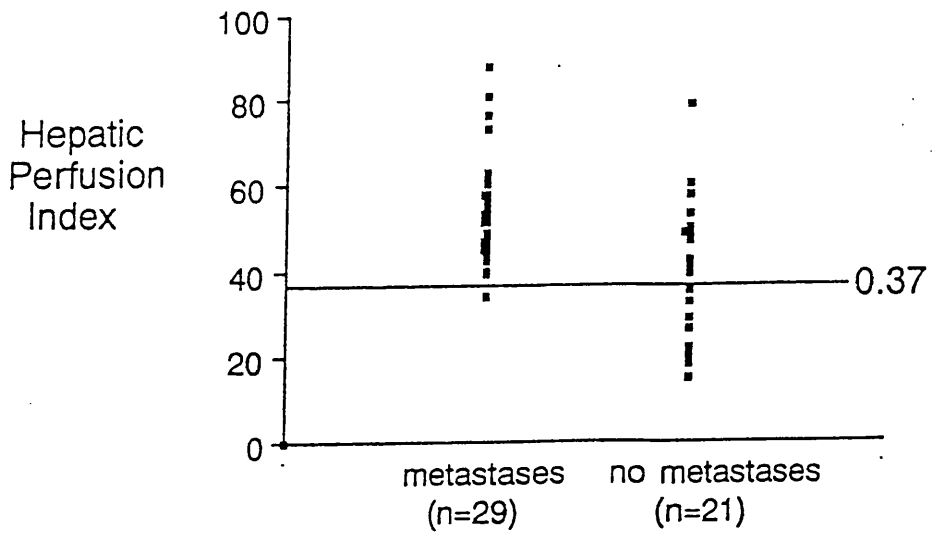


Figure 16 : Clinical correlation of the HPI in patients with colorectal carcinoma

(2)Comparative study of imaging techniques:

Seventy-three consecutive patients with colorectal carcinoma were studied.In 33 patients,one or more imaging modalities were omitted because of emergency presentation(n=14) or subsequent palliative non operative treatment(n=19) preventing intraoperative palpation or ultrasonography. Consequently percutaneous ultrasound, computerised tomography, hepatic scintigraphy, and the hepatic perfusion index,were performed on 73 patients, intra-operative palpation in 54,and intraoperative ultrasound in 40 patients.

Patients were considered positive if they had histological or post-mortem confirmation of metastases,or retrospective evidence of disease progression on serial followup imaging. Of the 73 patients 36(49%) were considered to have definitive evidence of hepatic metastases,the high percentage reflecting the Departments use as a referral centre. The relative clinical accuracy of these techniques in the detection of hepatic metastases are summarised below and detailed in Appendix 3.

The comparative study was limited to those patients in whom all techniques had been employed(n=40). One dynamic hepatic scintigraphic study was unable to be processed due to focal uptake. Computerised tomography and dynamic scintigraphy were the most sensitive imaging techniques,both detecting over 90% of hepatic lesions. Real time ultrasonography was disappointing, having a sensitivity of only 77%. These scans were performed on routine lists this may simply reflect inter-operator variability. Intraoperative ultrasound had a sensitivity of only 77% in the detection of metastases,but with 100% specificity. In two cases,because superficial metastases were easily palpable,it was considered inappropriate to

attempt to visualise these lesions through an "aqueous window",which would have increased the recorded sensitivity. The other two missed lesions were small metastases near the bare area of the liver. The sensitivity of intraoperative palpation(82%) was higher than expected and must reflect an unusually high number of superficial metastases.

Table 7: Prospective imaging study

Comparative Study(n=40)

IMAGING TECHNIQUE	SENSITIVITY	SPECIFICITY	+VE PRED. VALUE	-VE PRED. VALUE
Ultrasound	(13/17)77%	(23/23)100%	(13/13)100%	(23/27)85%
CT	(16/17)94%	(21/23)91%	(16/18)89%	(21/22)95%
Static scan	(15/17)88%	(19/23)82%	(15/19)79%	(19/21)90%
Dynamic scan	(16/17)94%	(8/22)36%	(16/30)53%	(8/9)89%
Palpation	(14/17)82%	(23/23)100%	(14/14)100%	(24/26)92%
Intraop.U/S	(13/17)77%	(23/23)100%	(13/13)100%	(24/27)89%

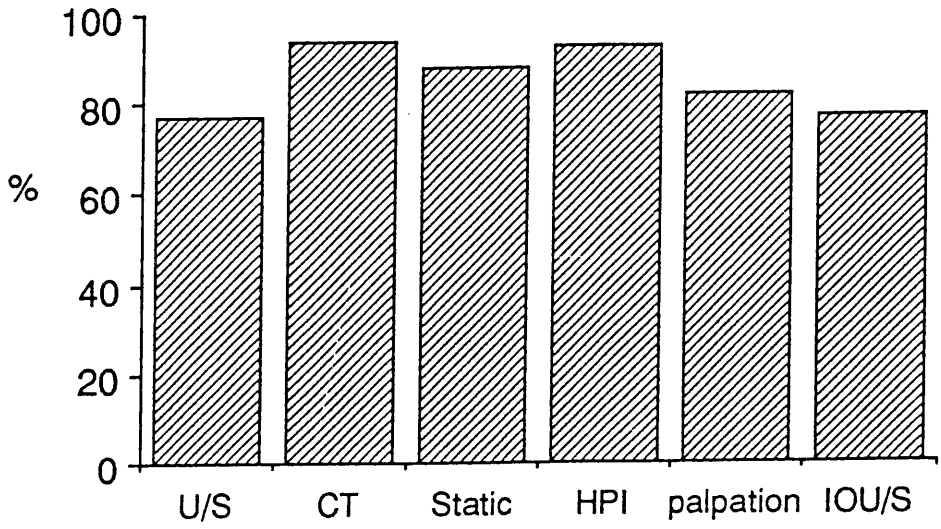


Figure 17: Sensitivity of modalities of imaging

Specificity in all tests apart from the HPI was at least 81%, indicating a hesitancy to report possible metastases. The low recorded specificity of the HPI may reflect its reported ability to predict the occurrence of metachronous metastases. If this is so, with time, the specificity of the HPI will improve,with a corresponding reduction in the sensitivity and specificity of the other techniques. It will therefore be several years before the prognostic value of an abnormal HPI in these apparently disease free patients can be accurately assessed.

Table 8 demonstrates the effect of the inclusion of the results of the other 33 patients in whom one or more of the tests was not performed,and it is apparent that this does not significantly alter the relative accuracy of each test. This further strengthens the findings of this study.

Table 8 : Comparison of imaging techniques including patients that did not undergo all imaging techniques(n=73):

IMAGING TECHNIQUE	SENSITIVITY	SPECIFICITY	+VE PRED. VALUE	-VE PRED. VALUE
Ultrasound	(27/36)75%	(37/37)100%	(27/27)100%	(38/46)83%
CT	(34/36)94%	(34/37)92%	(34/37)92%	(34/36)94%
Static scan	(29/36)80%	(31/37)81%	(29/36)80%	(31/37)84%
Dynamic scan	(34/36)94%	(16/36)44%	(34/54)63%	(16/18)89%
Palpation	(23/25)92%	(29/29)100%	(23/23)100%	(29/31)94%
Intraop.U/S	(13/17)77%	(23/23)100%	(13/13)100%	(24/27)89%

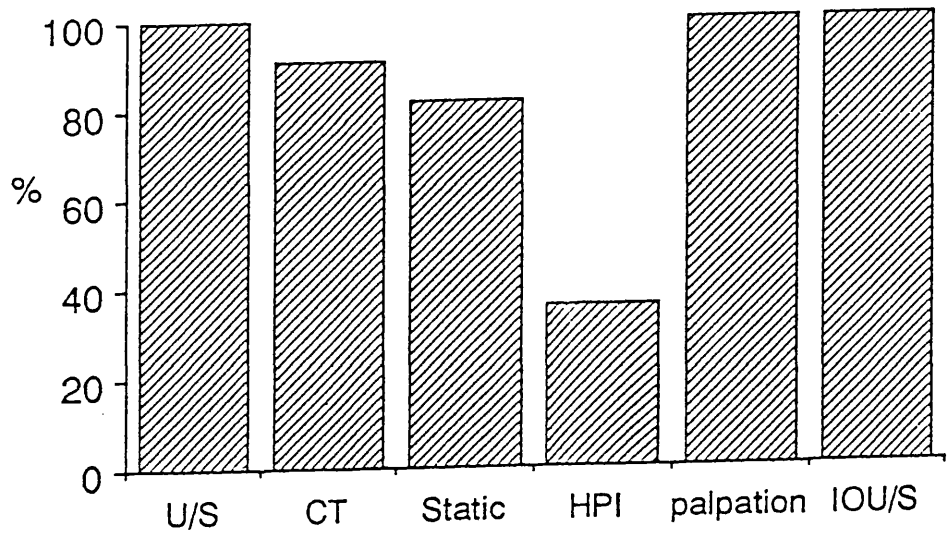


Figure 18 : Specificity of modalities of imaging:

SECTION 2

ANIMAL MODELS AND HAEMODYNAMIC STUDIES

The HSN sarcoma cell tumour model:

Prior to inoculation the viability of the cells was confirmed by the addition of trypan blue. Tumour was achieved in all animals undergoing inoculation with live cells. Intrahepatic tumour deposits were usually undetectable (median diameter=0mm(0-1)) at five days following induction,becoming more obvious between ten days (2mm(0-3)) and fifteen(4mm(2-7)). By twenty days 8mm(6-10) they entered their rapid growth phase,reaching 23mm(18-28) by twenty-five and 28mm(21-42) by thirty days.

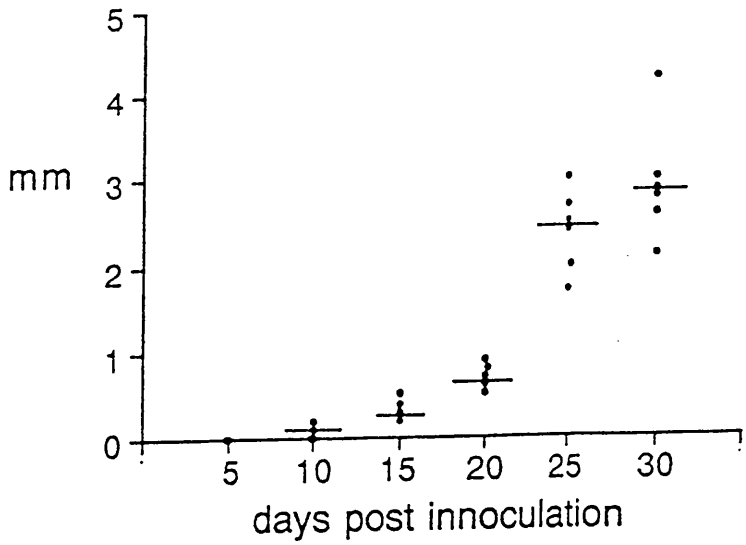


Figure 19: Growth curve of cell line.

All experiments were performed between 20 and 25 days following inoculation to correspond with the rapid phase of the growth curve when tumours of between 1-3 mg were present. The median % hepatic replacement of these tumours at this stage was 15.6% (range 2 - 42%) (See Appendix 4). These tumours are relatively hypovascular with a median blood flow to liver tissue, as determined by the reference microsphere sample technique, of 0.124ml/min/g(range 0.095-0.159) compared to 0.085(0.045- 0.17) with a median tumour to liver blood flow ratio of 0.503 (0.28- 1.70).

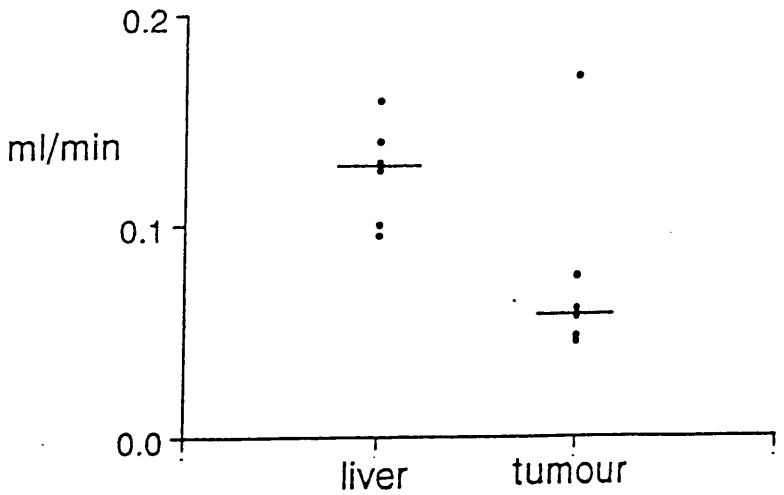


Figure 20: Relative blood flow of liver and tumour tissue.

Cross perfusion experiment:

As discussed earlier this was performed to attempt to prove that the alterations in splanchnic haemodynamics are as a result of a circulating,and therefore transmissible,vasoactive agent. A total of 62 experiments were performed. The initial experiments were plagued by instability created by the complexity of the model. Consequently more than two thirds of the experiments were principally concerned with overcoming technical problems and the development of a stable model,with physiological temperature (36-38^{o}C),electrolytes(Na^{2+} (130-145),K^{+} (3.0-5.0) ,Cl^{-}(90-105), Urea(<10),blood gases (pO_2(100-150mmHg) CO_2 (40-50 mmHg) and acid/base balance(pH(7.30-7.50).

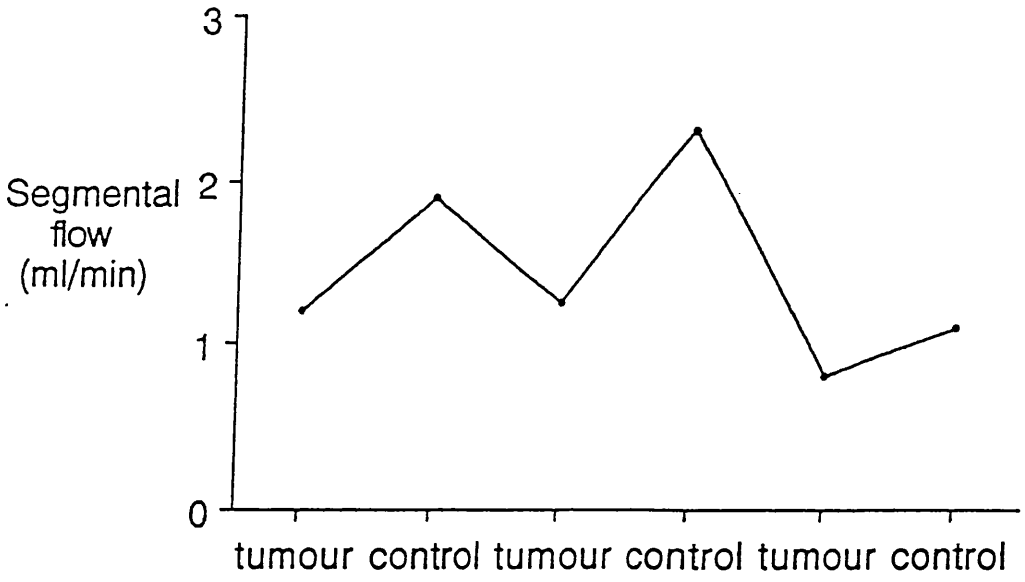


Figure 21: Segmental flow in experiment 1 during alternate periods of perfusion by tumour bearing and control animals

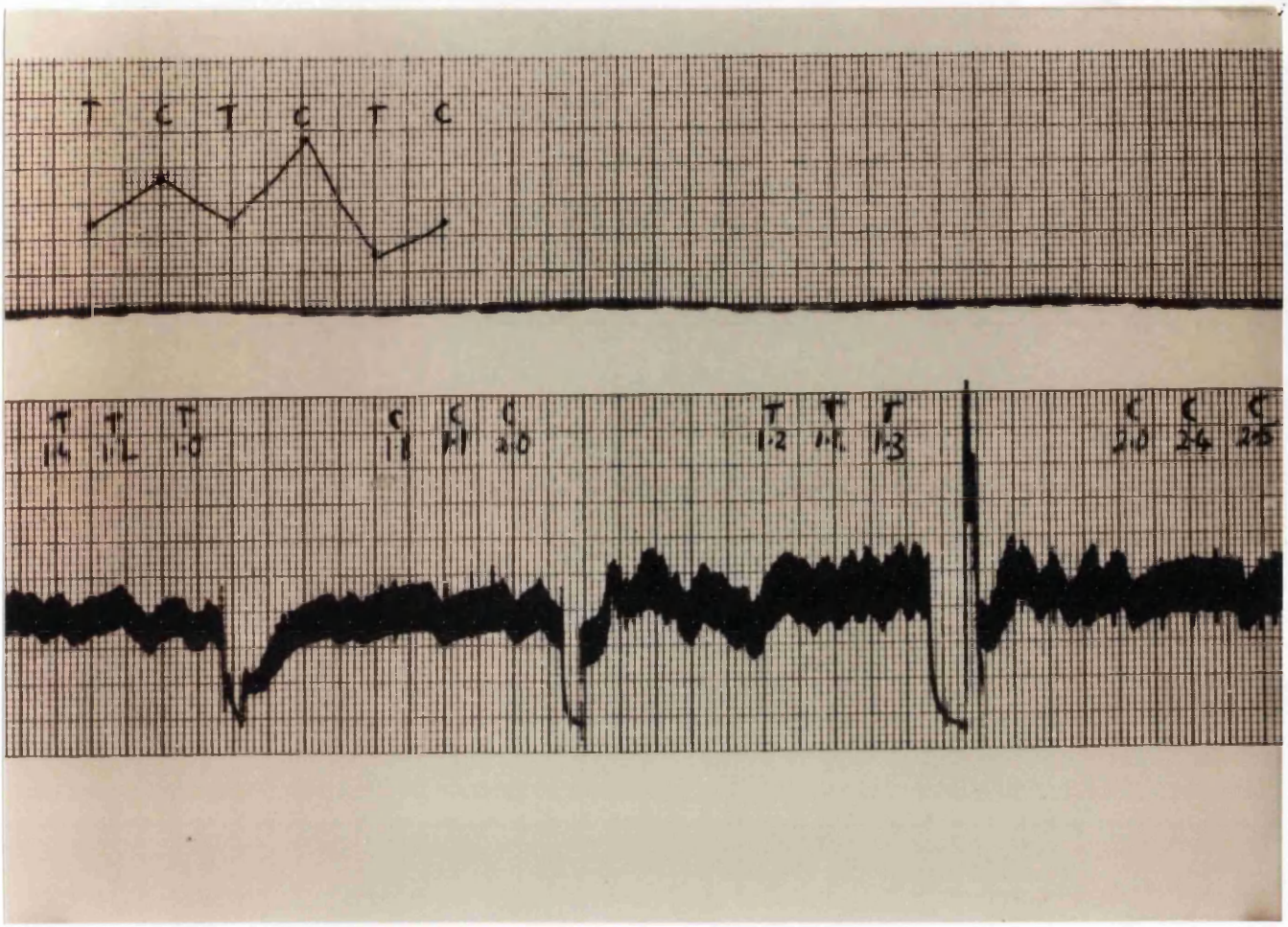


Figure 22: B.P. tracing and associated flows of experiment 1

This is a photograph of the continuous blood pressure tracing obtained during a cross-perfusion experiment. The arterial trace (having previously been calibrated) records the perfusion pressure developed by the donor animal. The figures refer to the simultaneous blood flow achieved through the isolated segment, during alternating periods of perfusion by tumour-bearing animals(T) and synergistic controls(C).

Cannulas reported to have been used in the literature(Anzueto, 1984) were found to produce unacceptably high resistance to flow and "tapering" cannulas from 2mm to 1 mm internal diameter were developed and used. Excessive fluid and temperature loss from the exposed small bowel was prevented by wrapping the segment with "clingfilm". Because of previously reported vascular homeostasis,only flows associated with a mean systolic blood pressure of between 80 and 130 mmHg were accepted.

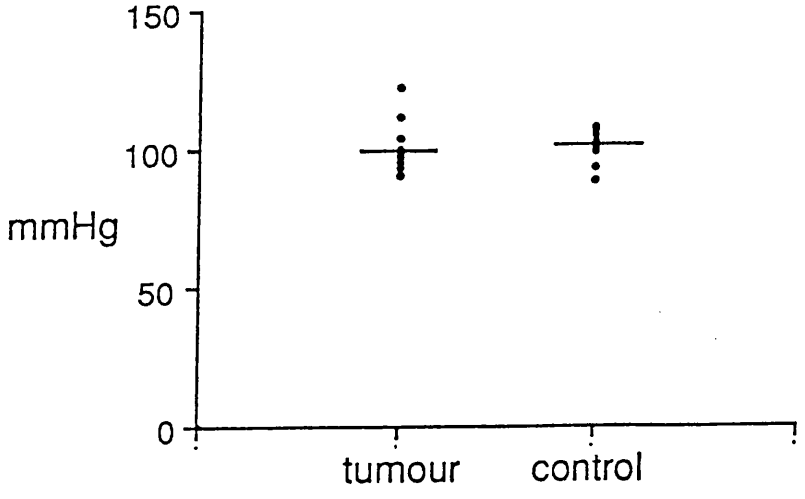


Figure 23: Blood pressure during perfusion by control and tumour rats:

The median arterial blood pressure in control animals was 101.7mmHg (range 90 - 108.3) as compared to 99.2 mmHg(range 90 -120.7) in the tumour bearing rats(p=0.82). The median flow through the small bowel segments was greater during perfusion by a control animal (2.1ml/min (range 1.33-3.93)) than during perfusion by a tumour bearing animal (1.58 ml/min (range 0.4-3.6))(p=0.12). The median calculated splanchnic vascular resistance was less in the during control perfusion (49.7 mmHg/(ml/min) (range 25 - 117) than during tumour perfusion (64.8mmHG/(ml/min)(range 26.9-243)(p=0.03).

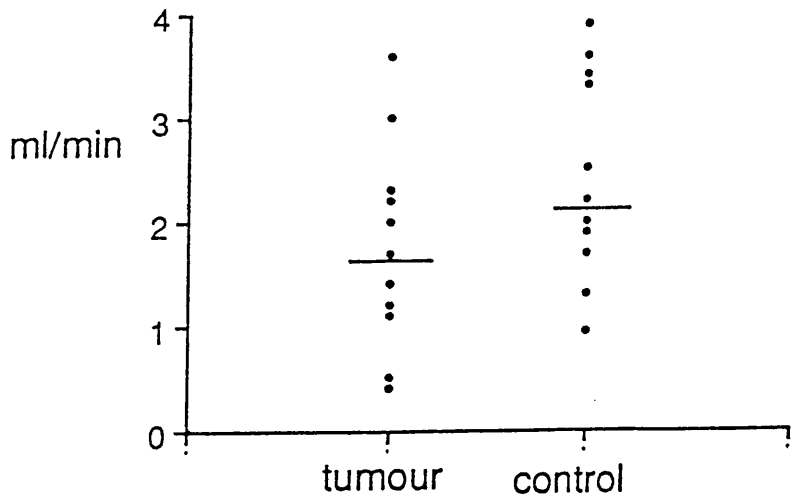


Figure 24: Segmental blood flow during perfusion by control and tumour bearing rats.

On the second cross perfusion (Appendix 3b) four experiments were terminated because the mean systolic pressure was not above 80mmHg. In the remaining animals the median systolic pressure was 100mmHg(range 85 - 110) compared to 100 mmHg (95 - 110)in tumour bearing animals(p=0.78). Once again the flow was greater (2ml/min (range 0.8 - 3.3) cf. 1.25(0.4-2.4))(p=0.32) and the resistance lower (51.2 (33-122.3) cf.76.1 (45.4-250)(p=0.13) during perfusion with control rats compared to tumour bearing animals.

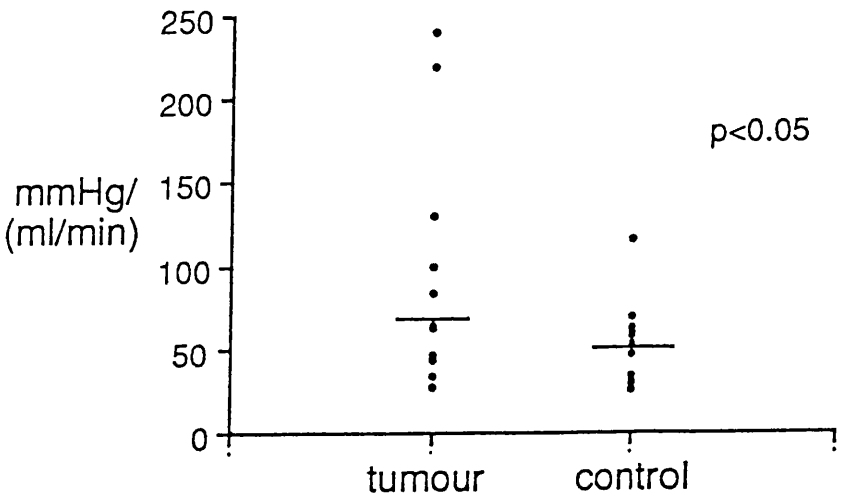


Figure 25: Mean resistance during perfusion by control and tumour rats

Reference microspheres:

In this section the results of the reference microsphere experiments are summarised and detailed in Appendix 4. This experiment was performed to investigate the effect of extrahepatic tumour on splanchnic haemodynamics.The mean recovered counts of the reference sample was 2830(range 1363-7878) with no significant difference between the three study groups. Tumour deposits were confirmed in two intrahepatic and flank sites in all animals inoculated with live cells.The livers in animals injected with dead cells showed only minimal fibrous scarring with no evidence of tumour.The mean tumour weight in animals with flank tumour was 1.75g(range 1.4-2.3) compared to 1.93g (range 1.2-2.6) in animals with intrahepatic tumour. Seventeen animals were excluded from analysis because the counts in the left and right kidney differed by more than 15%.

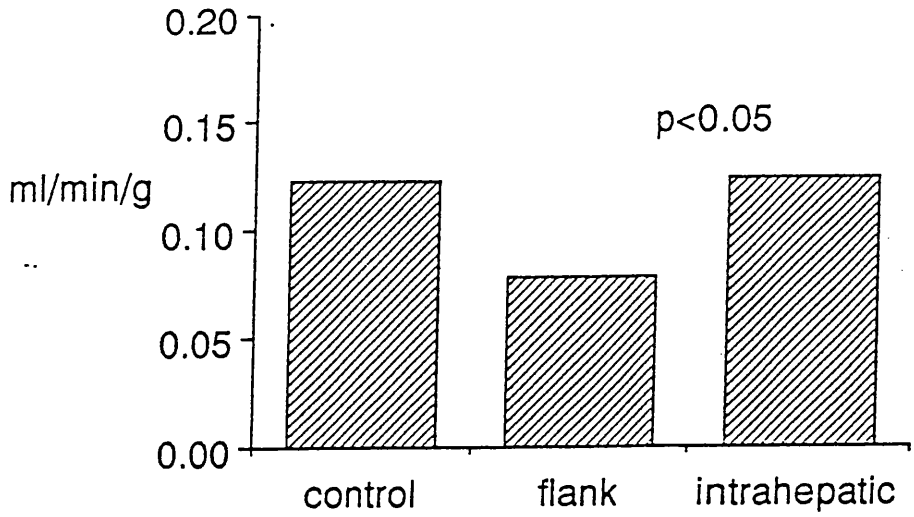


Figure 26: Flow per gram of liver

The calculated blood flow confirmed that,as in previous experiments intrahepatic tumour was hypovascular relative to surrounding liver parenchyma with a mean tumour to liver blood flow ratio per gram of tissue of 0.67 (range 0.33-1.7). When blood flow is calculated on a per gram basis, the hepatic arterial flow to liver parenchyma was similar in controls(0.123 ml/gm) and liver tumour bearing animals(0.124 ml/gm) but less in flank tumour bearing animals (0.078ml/gm). The flow per gram to the splanchnic organs was greater in control animals(0.789 ml/gm) compared to both liver tumour bearing (0.461 ml/gm) and flank tumour bearing (0.489 ml/gm) animals.

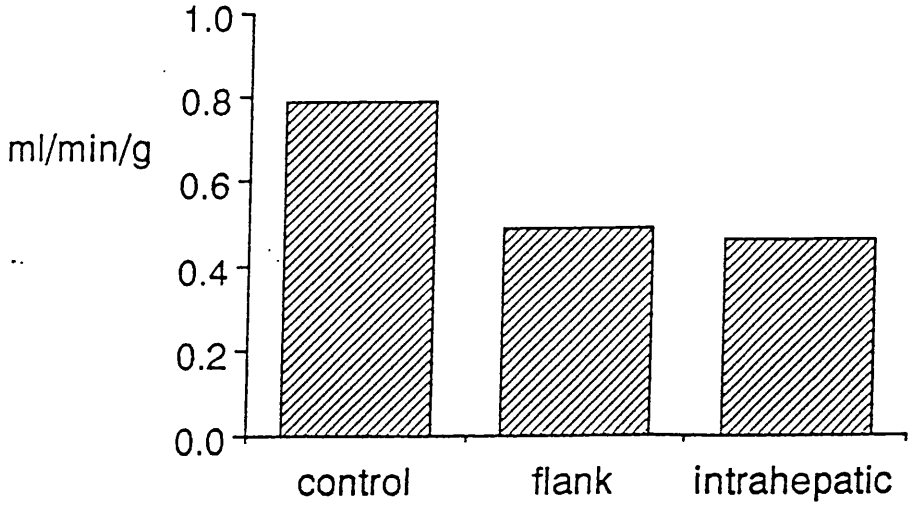


Figure 27: Flow per gram of splanchnic tissue.

Total blood flow to tumour bearing livers,including tumour blood flow, was greater than to either controls or animals with flank tumours(1.31 ml/min compared to 0.91 ml/min and 0.75 ml/min respectively). The splanchnic blood flow was reduced in both animals with tumour in the liver and flank(7.75 ml/min and 8.69 ml/min) relative to control animals (11.36 ml/min).

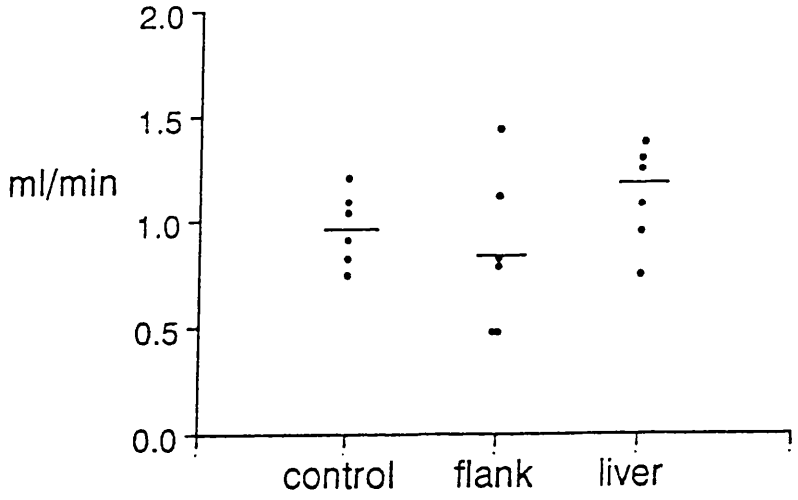


Figure 28: Flow to liver parenchyma in controls,and animals with tumour in the liver and flank.

The calculated Hepatic Perfusion Index,excluding tumour blood flow, was significantly increased in animals with tumours within the liver (0.11(0.09-0.19)) compared to animals with flank tumours (0.08 (0.07- 0.10)) and control animals(0.08(0.06-0.09). The inclusion of the flow to tumour in calculating the HPI(0.13 (0.11-0.23) in the intrahepatic group further increased the magnitude of the change.

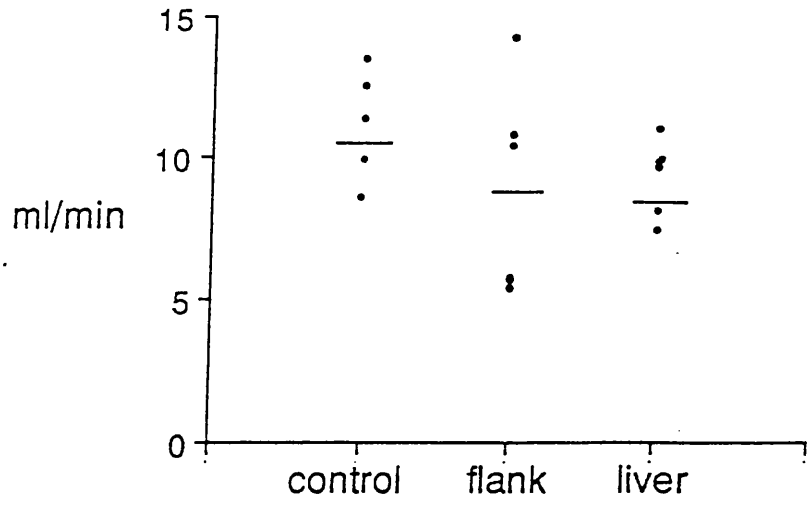


Figure 29: Flow to splanchnic organs in controls, and animals with tumour in liver and flank.

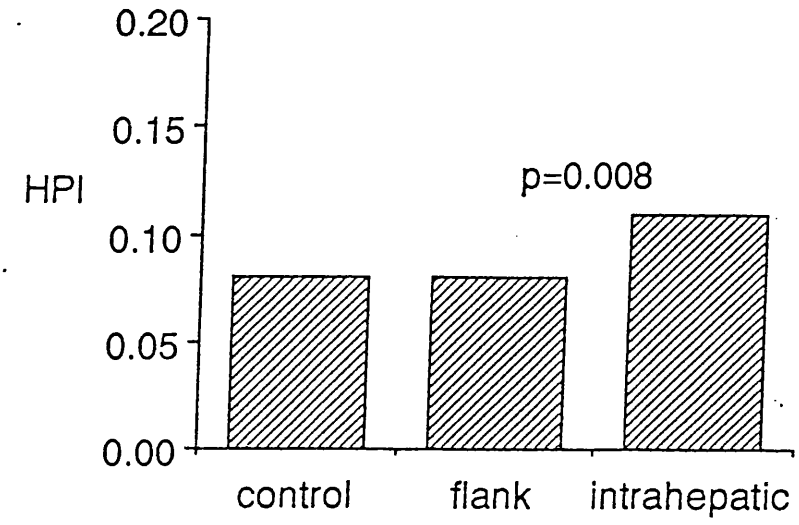


Figure 30: Calculated hepatic perfusion index for controls, and animals with tumour in liver and flank.

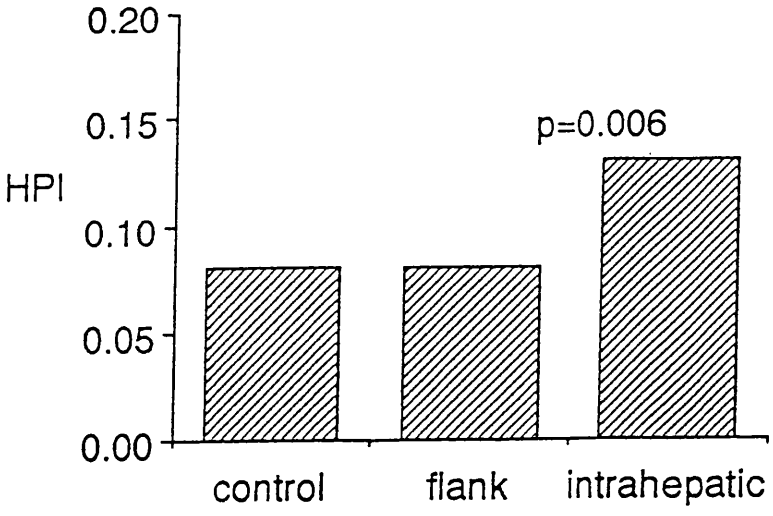


Figure 31: Calculated Hepatic perfusion index(incl. tumour blood flow)

SECTION 3

DRUG TARGETING

In this section I will first describe the results of experiments designed to assess the efficacy of three agents with potential use for hepatic targeting for adjuvant therapy and then the results of experiments looking at intrahepatic targeting in the presence of overt tumour.

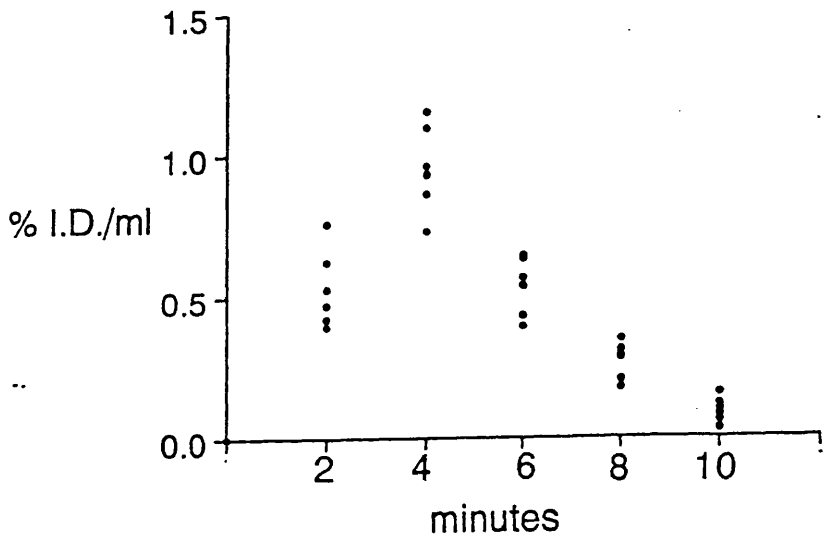
FIRST ORDER TARGETING

(1) 5-FU/GUARIC ACID POLYMER DISTRIBUTION STUDIES

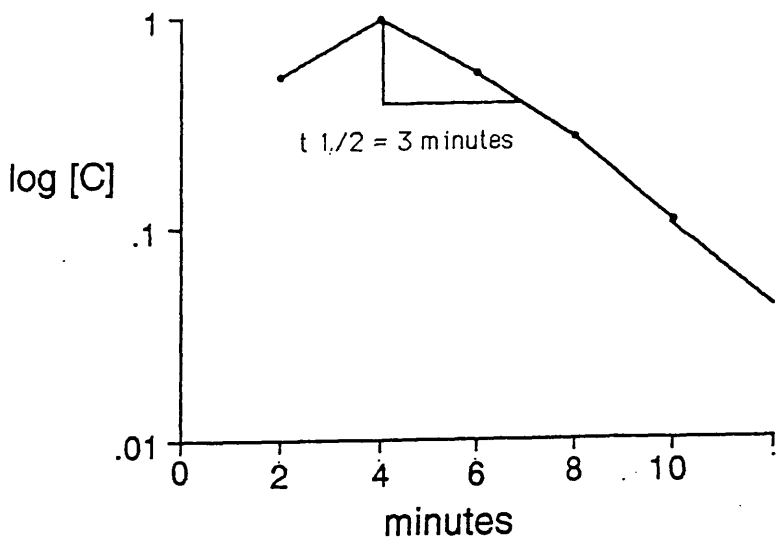
The results are summarised here and detailed in Appendix 5. A recovery experiment confirmed that the counts were unaffected by the volume of dissolved tissue used(Appendix 5a). Venous pharmacokinetic sampling following an intravenous bolus injection of free 5-FU acetate confirmed a rapid plasma peak(t_{max}= 4min) followed by a rapid fall($t_{1/2}$ = 3 mins) to a low concentration, indicating a large volume of distribution. A similar pattern was seen following injection of the 5 FU polymer(t_{max}=4 mins),but with a slightly prolonged half life ($t_{1/2}$=7.5mins),indicating a little,if minimal,retention within the circulation.

Using the area under the plasma concentration curve for each animal,the median AUC for 5-FU(4.74 %I.D.$ml^{-1}.hr^{-1}$) and 5-FU polymer(6.21 %I.D..$ml^{-1}.hr^{-1}$) treated animals was calculated,providing an AUC ratio($AUC_{5\text{-}FU}$:$AUC_{polymer}$) of 1.309,indicating slightly increased total drug exposure using the polymer.

Figure 32: Venous pharmacokinetics following bolus injection of 5FU acetate

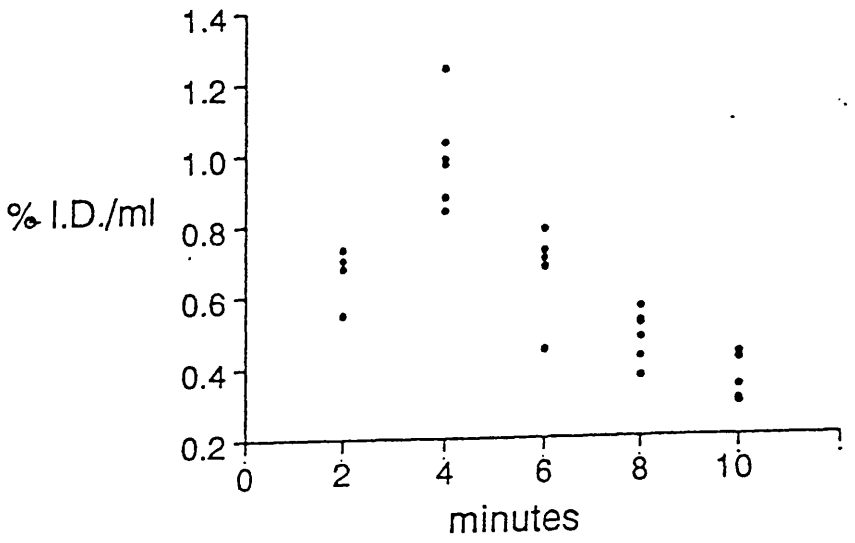


(1) median % injected dose/ml[C] vs time

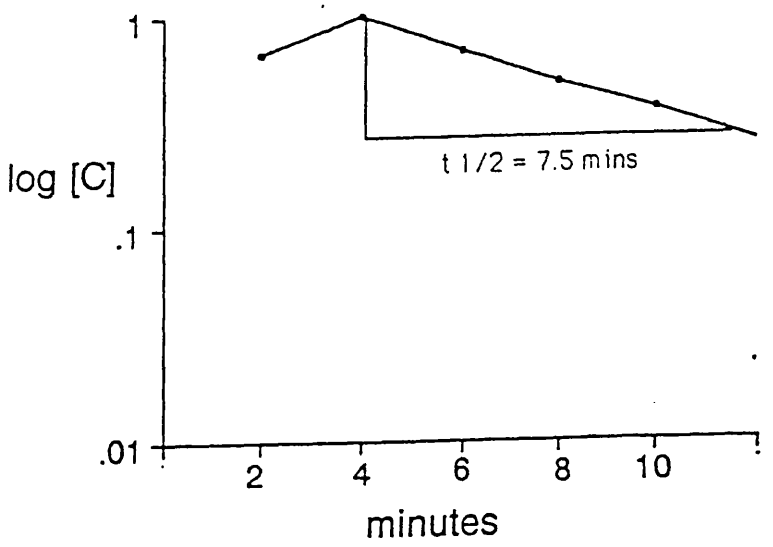


(2) log median [C] vs time

Figure 33: Venous pharmacokinetics following injection of 5FU/Guaric acid polymer



(1) median % injected dose/ml[C] vs time



(2) log median [C] vs time

There was a minor element of hepatic targeting,however only 7% of the injected dose was detectable in liver at 60 minutes (compared to 4.5% with free 5-FU acetate). Less than 0.5% of the injected dose was detectable in tumour tissue,and this was not greatly altered by the compound used.(Acetate(0.32%) cf polymer(0.24%). Tissue levels per gram of tissue were highest in kidney tissue,being over four times greater than in liver tissue,and over 20 times greater than in tumour. Due to the minor degree of hepatic targeting,the tumour : liver ratio was worse using the polymer than free acetate.

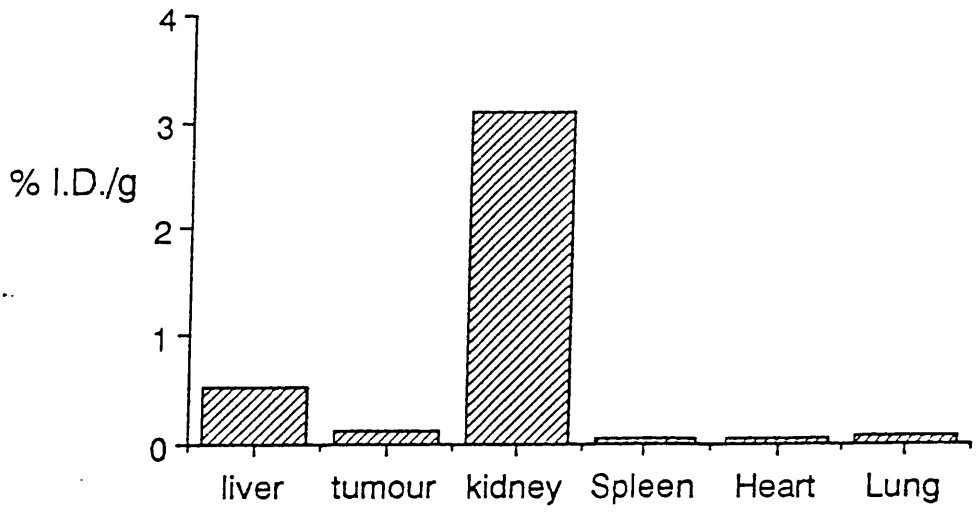


Figure 34: Distribution of 5 FU acetate(median % Injected dose/g)

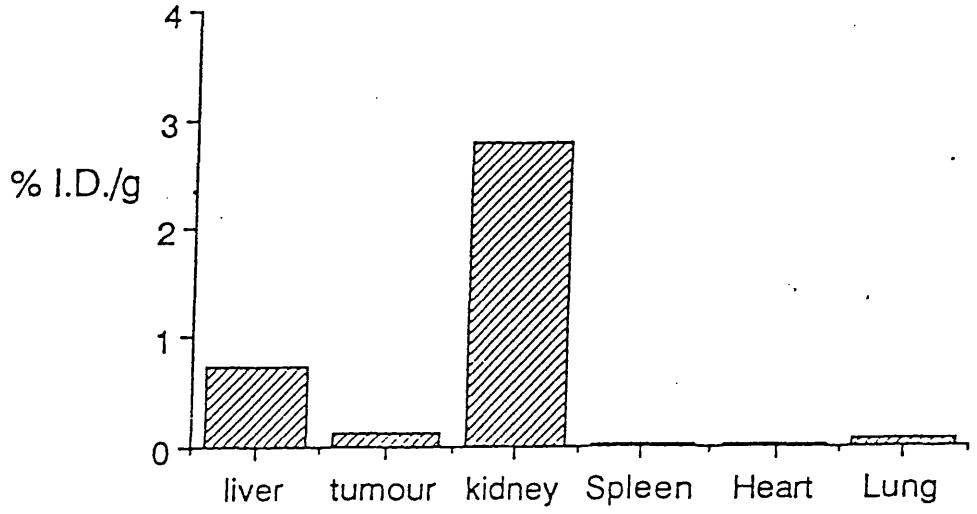


Figure 35: Distribution of 5FU/Guaric acid polymer(median % inj. dose/g)

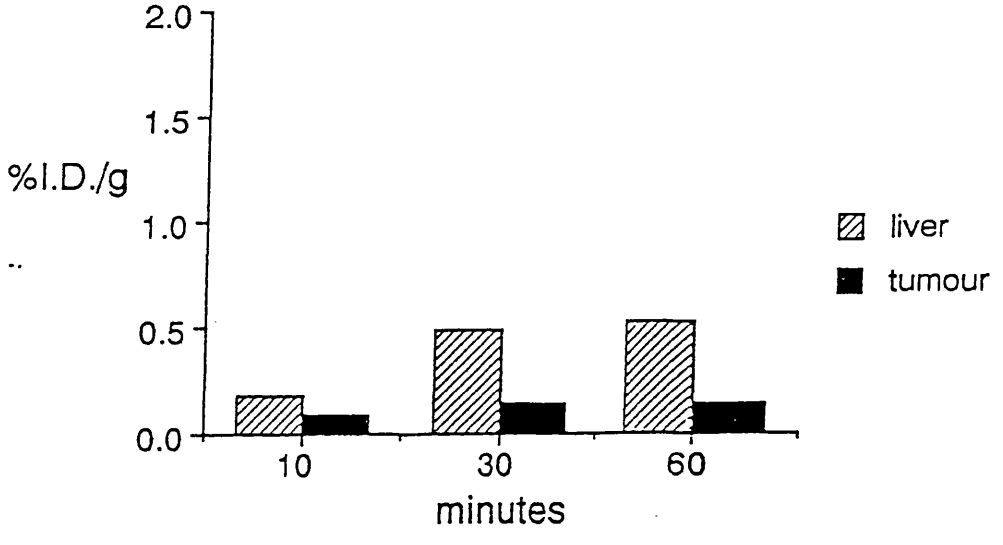


Figure 36: Tumour:liver ratios at 10,30 and 60 minutes following injection of 5FU acetate

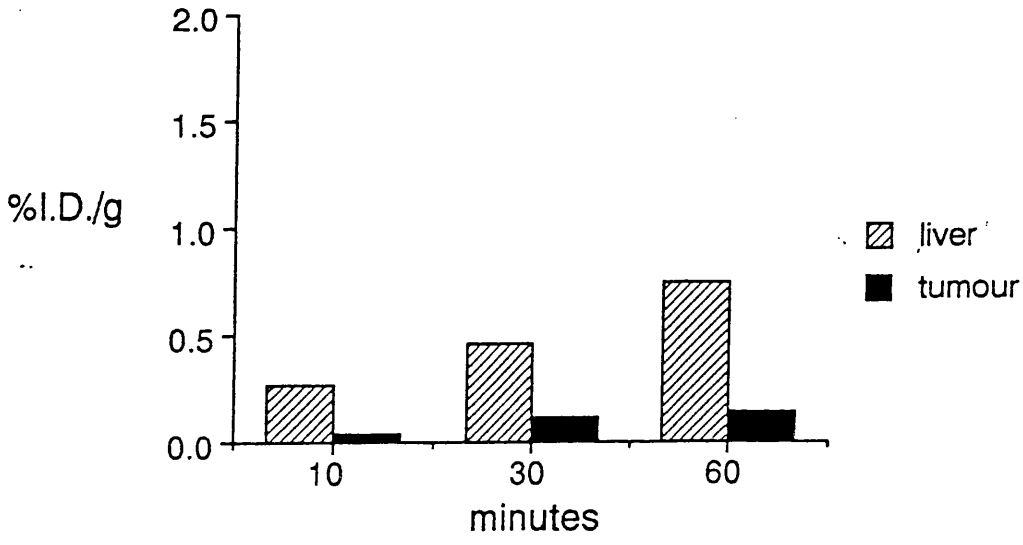


Figure 37: Tumour:liver ratios at 10,30 and 60 minutes following injection of 5FU/Guaric acid polymer

(2) N-(2-HYDROXYPROPYL)METHACRYLAMIDE POLYMER

Initial recovery experiments comparing the reliability of the estimation of the injected dose when administered through polyethylene cannulae and needle,showed considerable variation and a mean recovered cannula to needle dose ratio of 0.57:1, indicating a degree of adherence of the polymer to the internal surface of the cannula. Consequently it was considered inappropriate to calculate the injected dose from a reference sample and all distribution experiments were calculated as a percentage of the recoverable dose in the animal.

Intravenous administration:

The venous pharmacokinetic profile following an intravenous injection revealed a t_{max} of approximately 45 seconds, with a $t_{1/2}$ of 3 minutes. The median AUC_{IV} was 1.655%R.D.ml^{-1}.hr^{-1}. Total body distribution confirmed targeting to hepatic parenchymal tissue, with a median of 55.8%(range 37-67%) of the recovered dose being present in liver at 5 minutes, rising to 65% (range 56-76%) at 10 minutes,thereafter remaining steady(figure 38). Median renal concentration at 5 minutes(6%) rose minimally to 6.5% at 30 minutes.

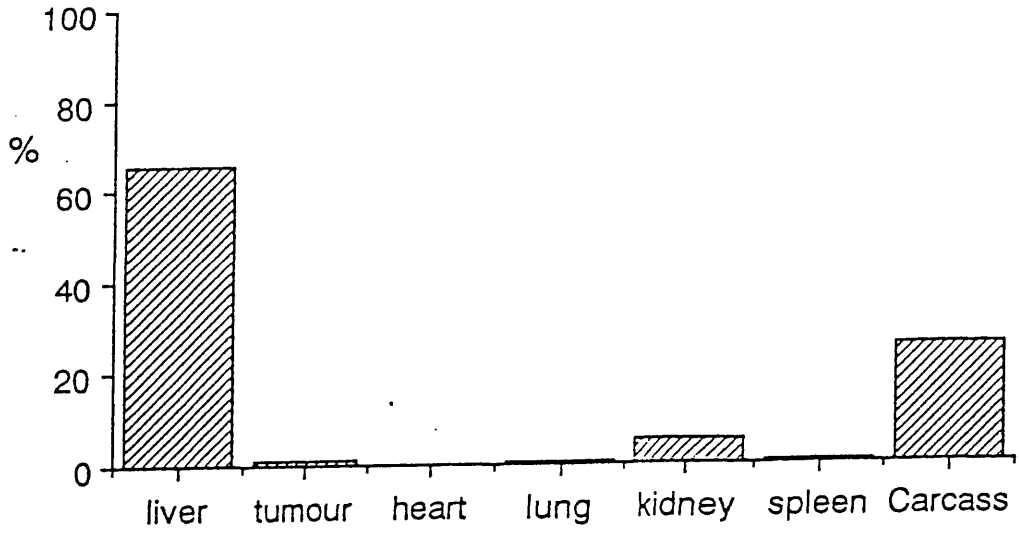


Figure 38: Polymer distribution following intravenous administration (median % I.D.)

Despite the excellent targeting to hepatic parenchyma, localisation in tumour was disappointing with a median 1.2% of the polymer being recovered from tumour tissue,the concentration being similar at 5 minutes (1.18(0.47-1.74)), 10 minutes (1.22(0.51- 2.85)) and 30 minutes (1.09(0.15-2.1)) following injection.

When expressed as a percentage of the recovered dose per gram of tissue the highest concentration of polymer was in liver with a median concentration of 5%/g at 5 minutes, increasing to 5.5%/g at 30 minutes. The concentration in kidney was approximately half this(2.4%/g increasing to 2.7%/g). Tumour tissue concentration was 0.27%/g increasing slightly by 30 minutes to 0.36%/g giving a median tumour to liver ratio of 0.07 : 1.

Regional administration:

The effect of administering the polymer via the hepatic artery or portal vein on systemic pharmacokinetics and whole body distribution was studied. There was no significant difference in the pharmacokinetic profile following either intraarterial or intraportal administration, presumably reflecting a low "first pass" uptake by the liver. Maximum serum concentration of polymer (t_{max}) being obtained at 45 seconds following injection with a similar half life($t_{1/2}$) of three minutes(figure 39). Comparison of the median AUC_{IA}(1.527%R.D.ml^{-1}.hr^{-1})and AUC_{IP} (1.609%R.D.ml^{-1}.hr^{-1}) with that following intravenous administration produced an AUC_{IV}:AUC_{IA} ratio of 1:0.92 and an AUC_{IV}:AUC_{IP} of 1: 0.97,indicating slightly less systemic total drug exposure following regional administration.

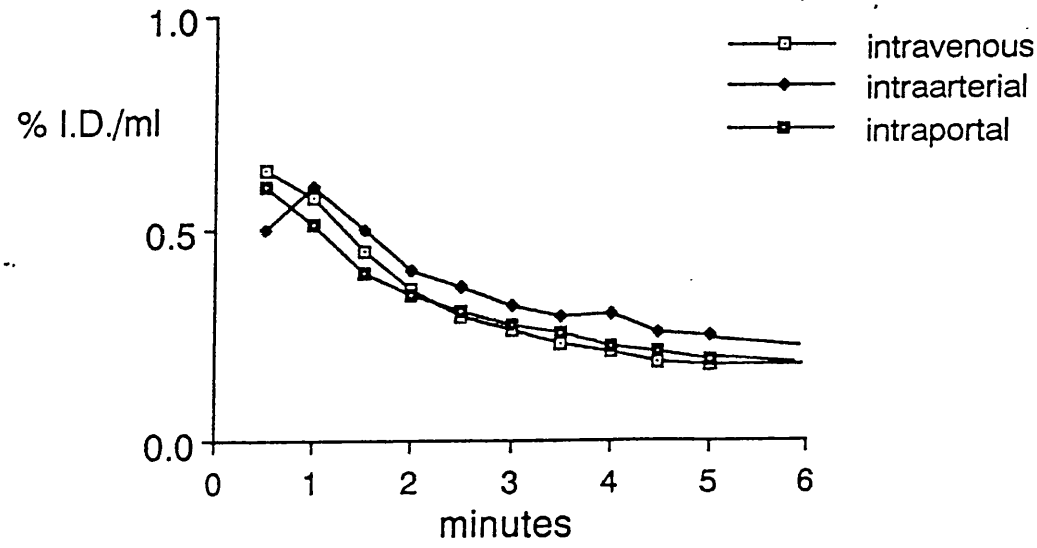
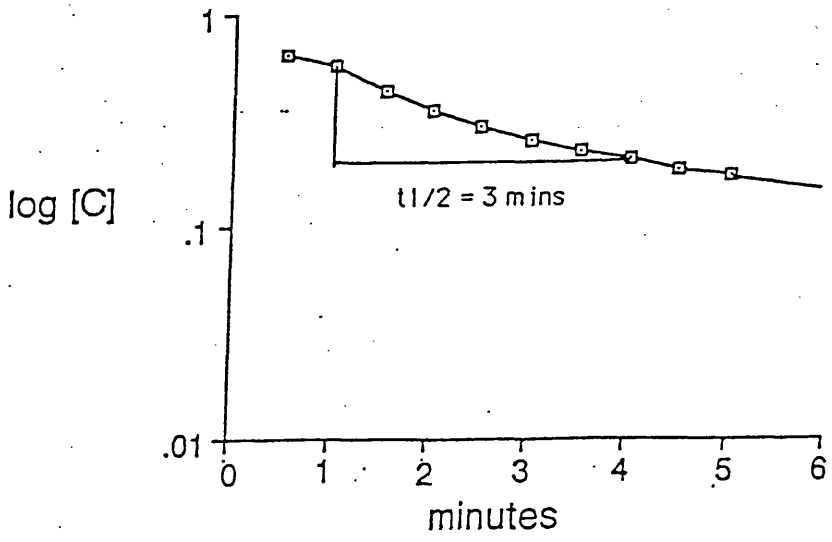
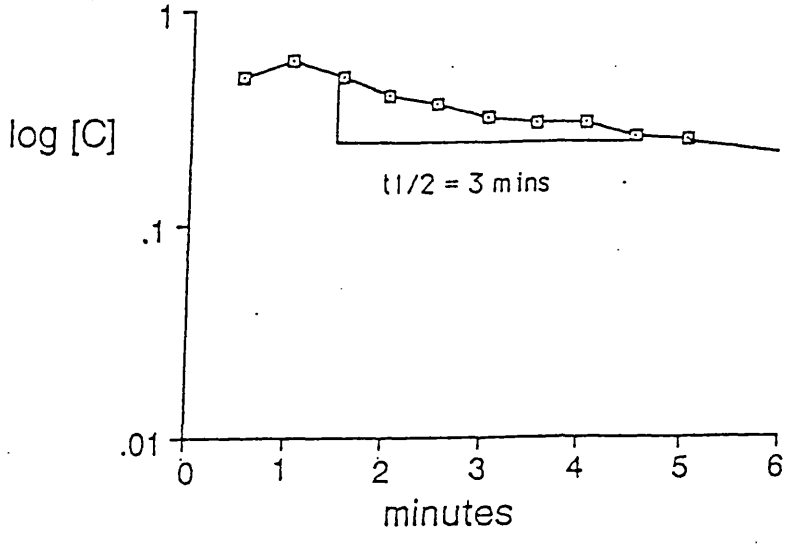


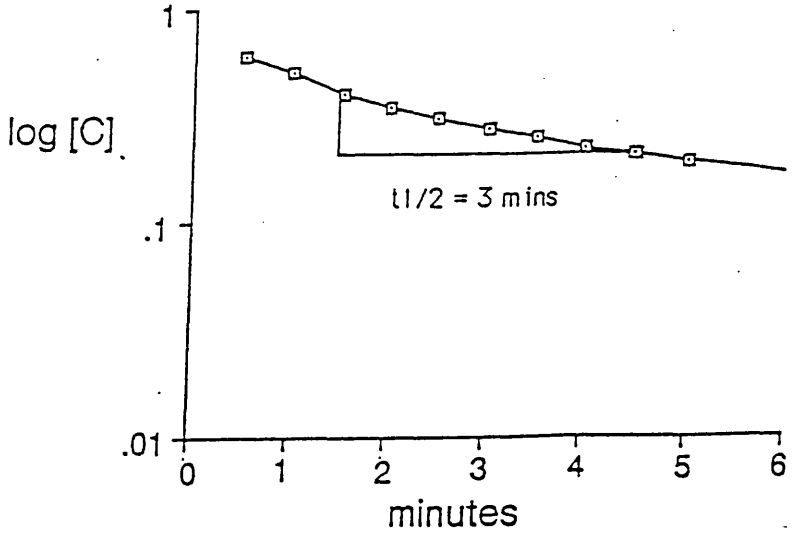
Figure 39: Venous blood concentration following intravenous, intraarterial and intraportal HPMA polymer administration(median % I.D./ml)



Intravenous



Intraarterial



Intraportal

Figure 40: Log concentration against time following intravenous, intraarterial and intraportal HPMA administration.

Table 9: Median tissue levels following intravenous,intraarterial, and intraportal administration:

		5 minutes	10 minutes	30 minutes
Liver	i.v.	55.85	65.40	64.05
	i.a.	56.55	56.05	60.15
	i.p.	57.65	60.80	62.99
Tumour	i.v.	1.18	1.22	1.09
	i.a.	2.93	2.19	2.16
	i.p.	1.40	0.92	1.24
Heart	i.v.	0.50	0.34	0.21
	i.a.	0.81	0.67	0.50
	i.p.	0.77	0.65	0.40
Lung	i.v.	1.40	0.85	0.57
	i.a.	0.90	1.19	0.65
	i.p.	1.08	0.76	0.47
Kidney	i.v.	6.14	2.45	6.44
	i.a.	5.85	6.24	7.81
	i.p.	4.95	3.68	4.29
Spleen	i.v.	0.30	0.20	0.29
	i.a.	0.88	0.81	0.51
	i.p.	0.65	0.48	0.41
Carcass	i.v.	34.70	27.60	26.65
	i.a.	30.35	30.60	27.25
	i.p.	33.20	30.75	26.35

With regional administration,as can be seen in table 9, because of the similar systemic availability of the alternative routes of administration, polymer distribution was not significantly affected by intraarterial or intraportal administration. Once again there was excellent hepatic targeting, nearly 60% of the labelled polymer localising within the liver in 5 minutes. The levels detected in kidney,spleen,heart, lung,and carcass(figures 41 and 42) were similar to intravenous administration.

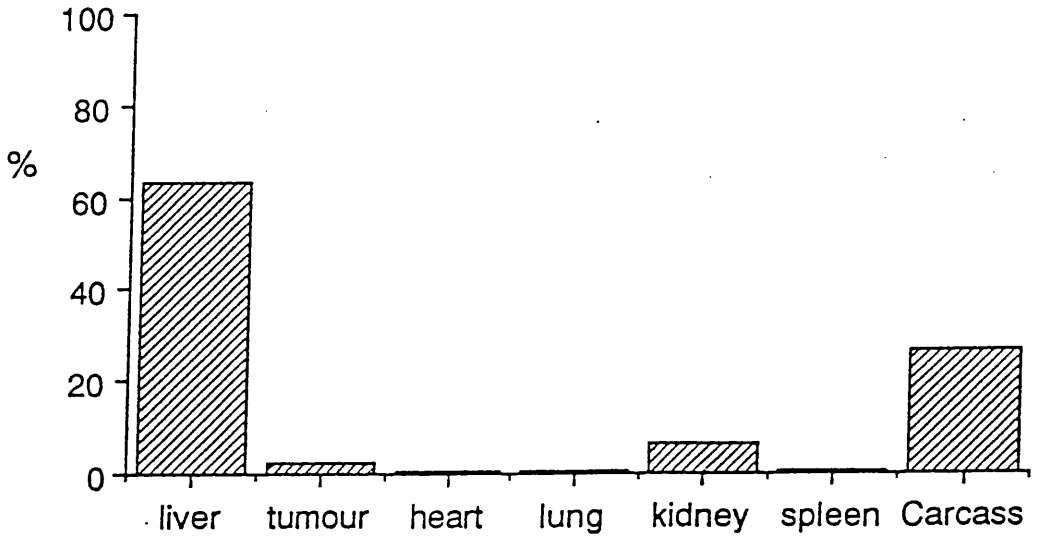


Figure 41: Polymer distribution following intraarterial administration (median % I.D.)

When expressed as a percentage of the recovered dose per gram of tissue, the levels in liver parenchyma(approx 6%/g) were over twice that of renal tissue(2.5%/g) and ten times that of heart (0.5%/g)and lung(0.5%/g). The relative concentrations obtained in tissue were unaffected by the mode of administration. (Appendix 5b)

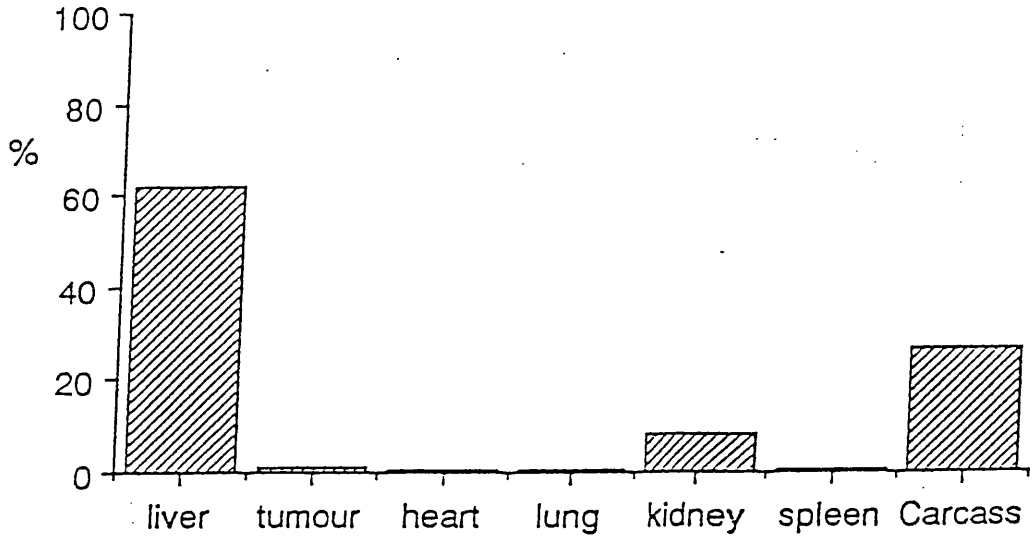


Figure 42: Polymer distribution following intraportal administration. (median % I.D.)

Tumour levels in all groups were disappointing.As a result of the preferential arterial delivery to tumour tissue, there was slightly higher concentration of polymer in tumour at 5 minutes(figure 43) following intraarterial administration(0.61%/g (0.25-1.34)) than by either intravenous(0.27%/g(0.16-0.96)) or intraportal administration (0.31%/g(0.26-0.67)) This difference was maintained at 30 minutes (0.74(0.18-1.26) cf 0.36(0.11-0.57)) and 0.32(0.21-1.54). Despite the improvement in polymer delivery to tumour,the median tumour to liver ratio was still only 0.12:1 (figure 44).

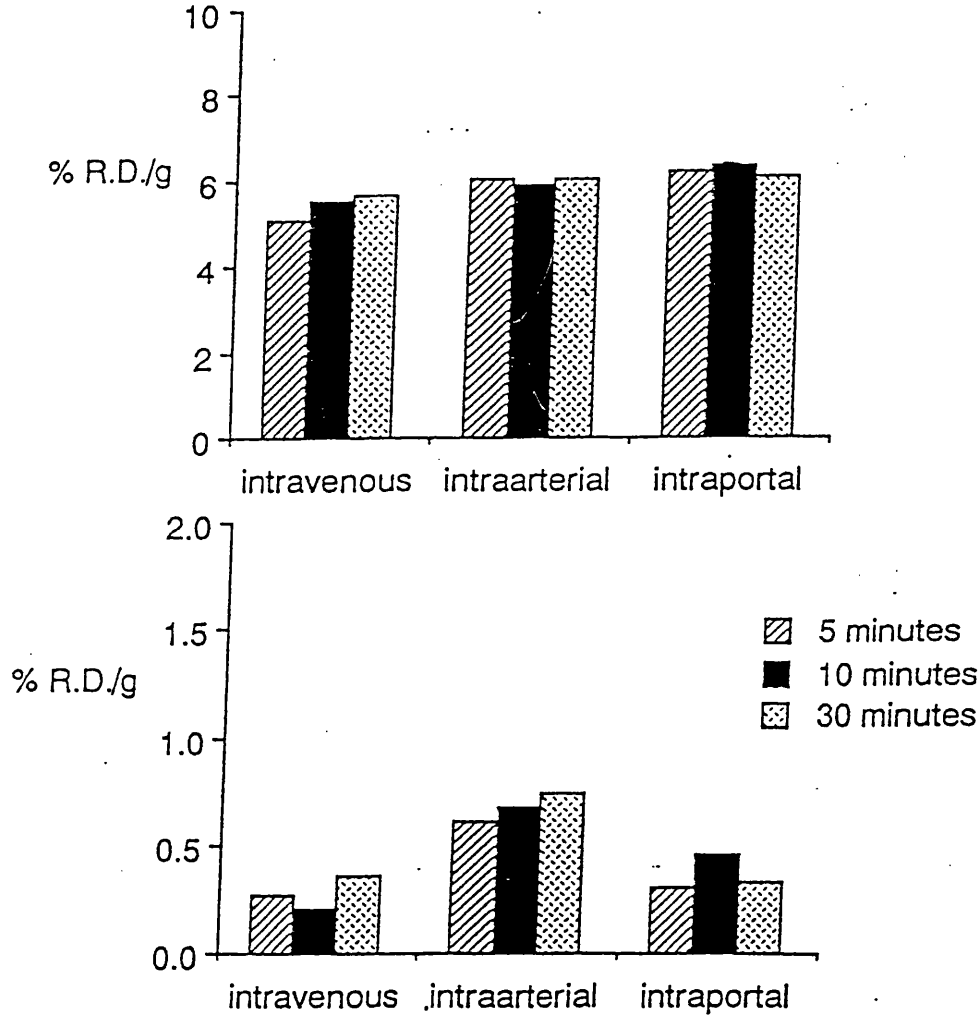


Figure 43: Uptake into tumour and liver parenchyma following intravenous, intraarterial and intraportal administration

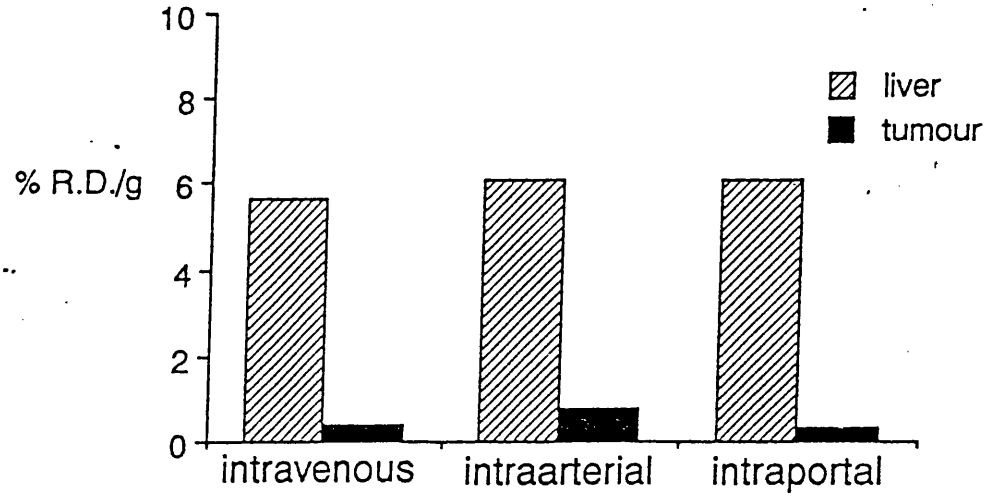


Figure 44: Tumour to liver parenchyma concentration ratio 30 minutes after intravenous,intraarterial,and intraportal administration

(3)^{14}C-LABELLED HAEMATOPORPHYRIN DERIVATIVE

The principal aim of this study was to determine firstly to what extent hepatic targeting occurred and secondly,whether the relative tissue concentrations varied with time;which would be useful in the temporal planning for the subsequent activation of the sensitiser. Localisation in the viscera was slow,58% of the recovered dose remaining in the carcass at 24 hours. There was progressive excretion of the radiolabelled porphyrin with 46% of the recovered dose on day 1 remaining by day 11.

The distribution pattern of the HpD was similar to the HPMA polymer,the Hpd being concentrated in the liver,spleen,kidney and tumour,only small amounts being recovered in the other organs. Localisation in the liver took 3 days to reach a maximum concentration. Thereafter the percentage of the recovered dose remained around 50% with a gradual reduction in absolute counts within the liver.

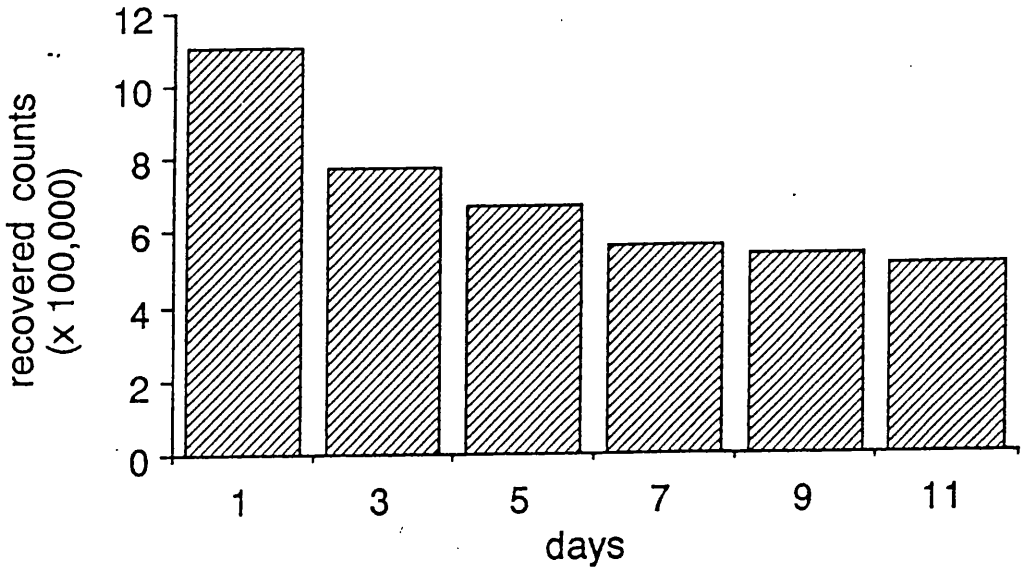


Figure 45: Total recovered dose with time.

By contrast,the uptake into tumour tissue was relatively rapid,the highest concentration(1.82 %/g) being recorded 24 hours following injection. Consequently,because of the more gradual concentration in liver parenchyma,the tumour : liver ratio was greatest on day one (0.75:1).

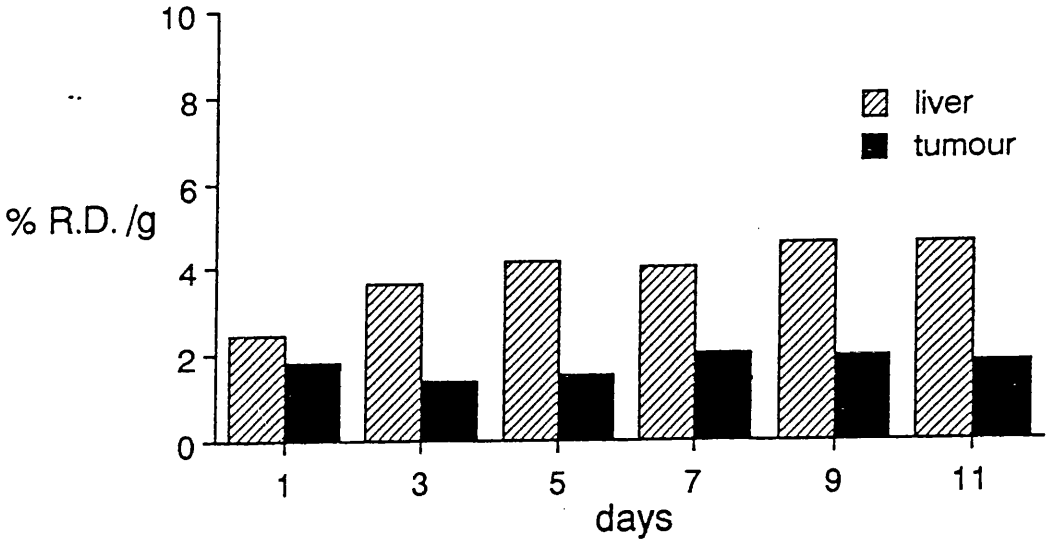


Figure 46: Tumour to liver ratios with time

When expressed as a percentage of the recovered dose per gram,the level detectable in the liver parenchyma remained steady throughout the study at 4%/g,which was slightly less than that of the HPMA polymer. There was also a gradual localisation within the spleen presumably due to progressive metabolism and excretion via the reticuloendothelial system.

Despite the early targeting to the hepato-portal viscera,a significant level of drug was detected in the carcass throughout the study,more than 30% remaining by day 11. This presumably reflects the prolonged photosensitisation experienced by patients receiving HPD.

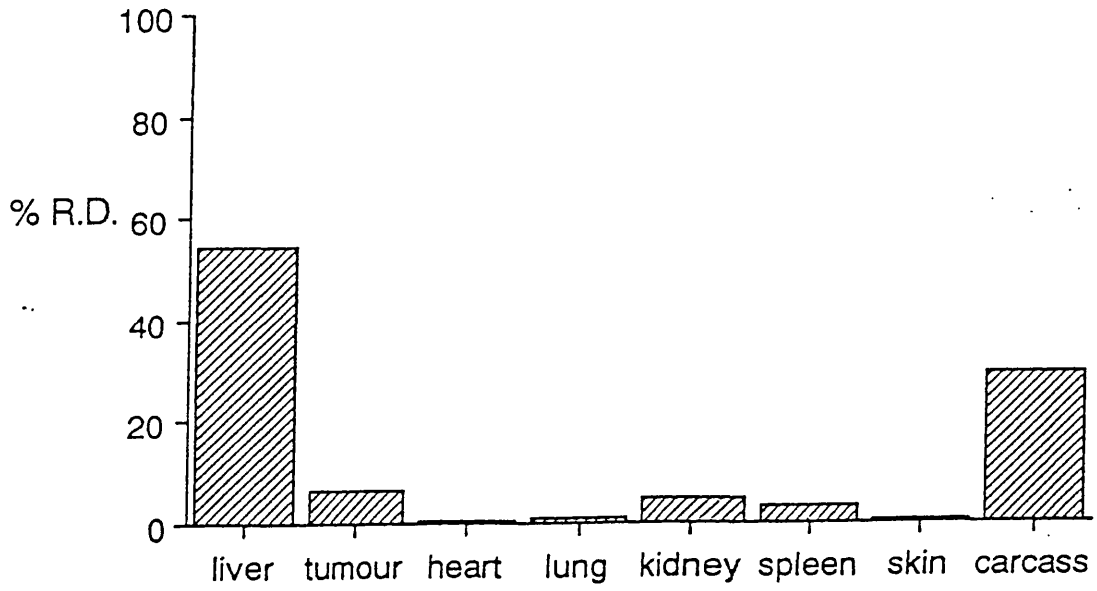


Figure 47: Distribution of ^{14}C-labelled Hpd at day 3

SECOND ORDER TARGETING

Pilot Study:

The results of the pilot study are illustrated below(figures 48 and 49) and detailed in appendix 6. This suggested that the most effective combination was achieved by the injection of Angiotensin followed one minute later by the degradable starch microspheres (2mg) and 30 μl of ^{99m}Tc methylene diphosphonate(MDP) given over 30 seconds. This schedule was therefore used in the comparative study.

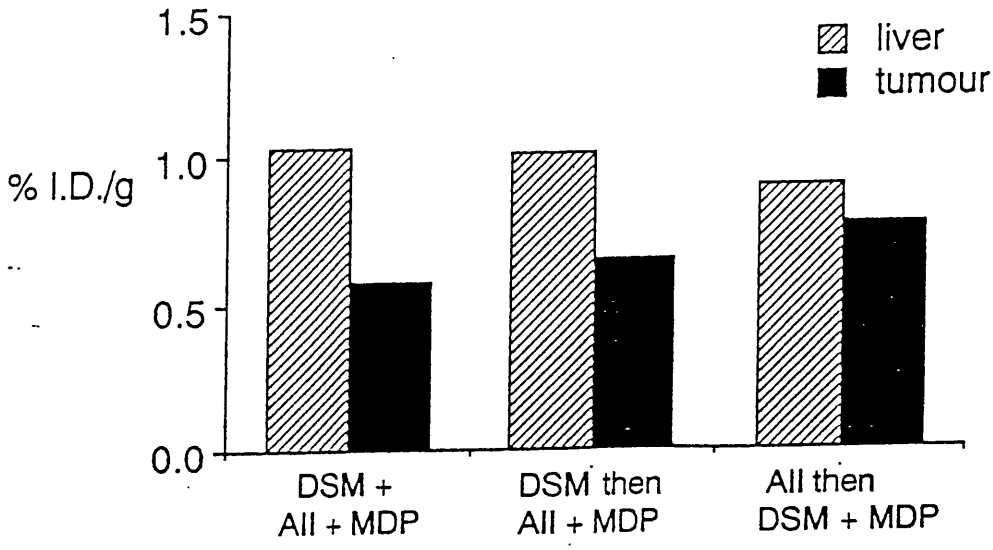


Figure 48: Pilot study. Liver and tumour distribution at one minute.

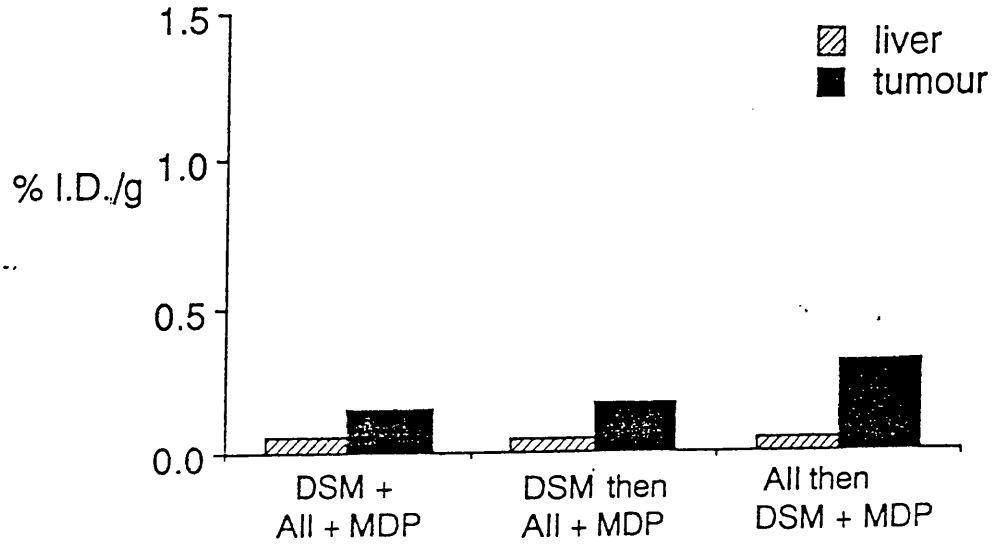


Figure 49: Pilot study. Liver and tumour distribution at 90 minutes.

Comparative study:

Comparison of the three study groups using the Kruskal-Wallis test confirmed that there was a significant difference in the retention of marker in tumour (p= 0.003) and liver (p= 0.001) at one minute,but only in tumour(p= 0,001) and not liver (p= 0.21) by 90 minutes. Whereas there was no significant difference in the tumour to liver ratio at one minute(p= 0.87) there was a significant difference by 90 minutes (p=0.001). Comparison between groups used the Mann-Whitney U-test for non-parametric data. The results are detailed in appendix 6 and summarised below. All results are expressed as the median(and range) of the percentage of the injected dose per gram of tissue.

ONE MINUTE:

Table 10: Uptake at one minute.(median(range))

	Control	DSM alone	DSM/Angiotensin
Liver parenchyma	0.08(0.01-0.62)	0.94(0.73-1.18)	0.95(0.4-1.01)
Tumour tissue	0.06(0.01-0.4)	0.71(0.09-4.55)	0.52(0.16-1.99)
T:L ratio	0.72(0.1-6.2)	0.69(0.1-4.2)	0.72(0.2-2.2)

Within control animals, the percentage of the injected dose of MDP per gram of tumour tissue was 0.06(0.01-0.4) which was lower than in the surrounding liver tissue(0.08(0.01-0.62)) but this difference was not statistically significant.($p=0.1$). The median tumour to liver ratio was 0.72(0.1-6.2).

In animals that were treated with DSM alone, the median uptake in normal liver tissue was 0.94(0.73-1.18) which was significantly greater ($p<0.001$) than in the control animals. Similarly the uptake in tumour tissue was significantly greater at 0.71(0.09-4.55) than in control animals($p=0.005$). The median tumour to liver ratio was 0.69(0.1-4.2) which did not differ significantly from that of the control group. ($p=0.87$).

Combining Angiotensin with the DSM again significantly increased the normal liver uptake to 0.95(0.4-1.01)($p=0.003$),and to 0.52(0.16 -1.99) ($p=0.003$) in tumour tissue compared to control animals. The median tumour to liver ratio of 0.72(0.2- 2.2)was again not significantly altered. Uptake achieved in both normal liver($p=0.49$) and in tumour tissue($p=0.79$) was not significantly different from animals treated with DSM alone.

90 MINUTES:

In control animals the level of marker remaining in normal liver tissue was 0.07(0.05-0.16) and in tumour tissue was 0.04(0.01-0.08) which was slightly less than in control animals at one minute due to washout of marker.There was relatively greater washout of marker from tumour tissue than in normal liver, with a median tumour to liver ratio of 0.49(0.2-1.1).

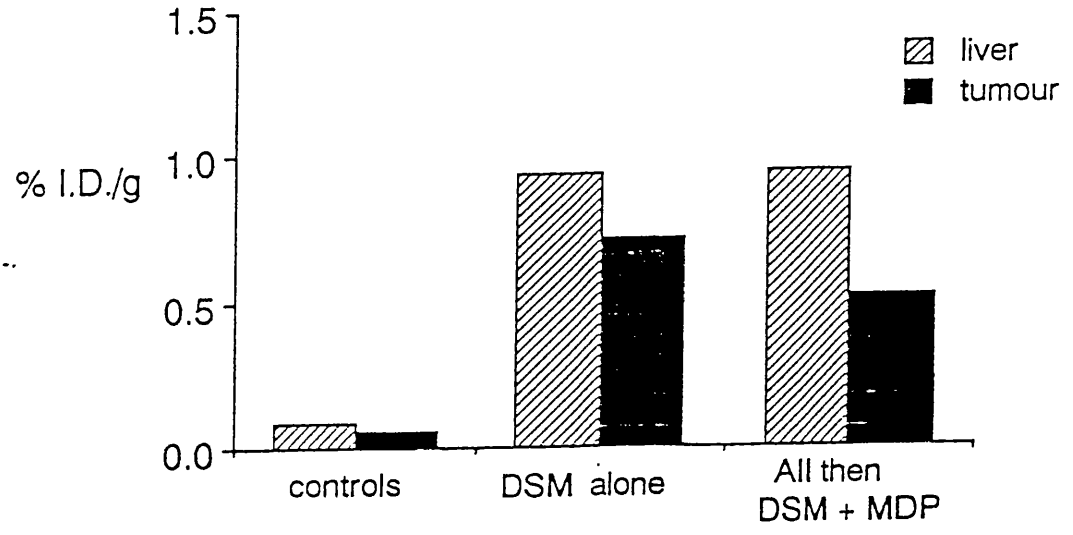


Figure 50: Uptake of marker at one minute following injection of saline,DSM, or angiotensin II followed by DSM

Table 11: Uptake at 90 minutes.(median(range))

	Control	DSM alone	DSM/Angiotensin
Liver parenchyma	0.07(0.05-0.16)	0.12(0.04-0.69)	0.11(0.03-0.24)
Tumour tissue	0.04(0.01-0.08)	0.19(0.06-0.75)	0.53(0.15-1.09)
T:L ratio	0.49(0.2-1.1)	2.09(0.1-3.6)	5.22(1.9-11.4)

In animals treated with DSM alone there was significantly increased retention of marker in tumour tissue(0.19(0.06-0.75)) ($p<0.001$)compared to control animals and also compared to surrounding liver tissue(0.12(0.04-0.69)($p=0.03$)) with a significantly increased tumour to liver ratio of 2.09(0.1-3.6). There was no significant difference in the retention of marker in normal parenchyma between DSM treated animals and controls at 90 minutes($p=0.1$)

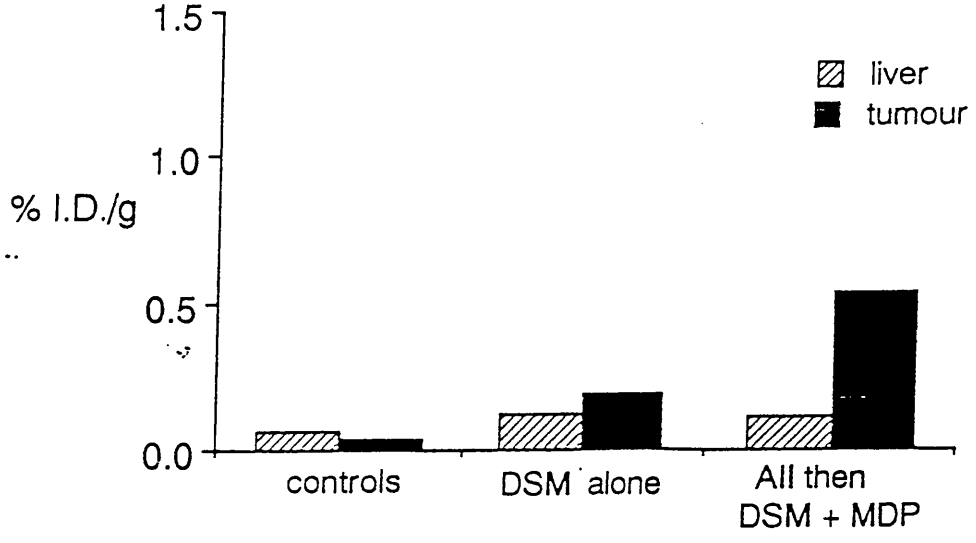


Figure 51: Uptake of marker 90 minutes following injection of saline,DSM or angiotensin II followed by DSM.

In animals treated with both DSM and angiotensin II, there was again significantly increased retention of marker(p=0.002) in tumour tissue (0.53(0.15-1.09)) compared to surrounding liver parenchyma(0.11 (0.03-0.24)). The tumour to liver ratio was also significantly increased (p=0.001) at 5.22(1.9-11.4) compared to both control animals and those treated with DSM alone. The absolute retention of marker in tumour tissue was also significantly greater than in controls ($p<0.001$) and DSM treated animals (p=0.01). The retention of marker in normal liver was not significantly different from that in control or DSM treated animals (p=0.64).

Blood pressure:

In animals treated with Angiotensin II the pretreatment median systolic blood pressure was 114mmHg(106-120).Following administration of the Angiotensin there was a rise in median systolic pressure to 131mmHg(120-143). One animal was excluded from further analysis when a rise in systolic pressure failed to occur.

SUMMARY OF FINDINGS

DETECTION OF METASTASES:

Section 1 - Clinical studies:

(1) Abnormalities in the hepatic blood flow pattern were detected in patients in patients with colorectal metastases.

(2) The high administered activity dynamic hepatic scintigraphy technique is reproducible when processed on more than one occasion by one observer or when separately processed by two independent observers.

(3) The HPI has a sensitivity of 94% in the detection of hepatic metastases. This is similar to computerised tomography and better than all other methods of detection. The specificity of the HPI ,in comparison to the other techniques, was poor(36%).

(4)The sensitivity of intraoperative ultrasound was disappointing (77%), although the specificity was excellent(100%). Intraoperative ultrasound only confirmed metastases that had been identified by other techniques, and was therefore found to have a similar threshold to standard techniques.

Section 2 - Animal models and haemodynamic studies:

(5) Hepatic tumour was reliably induced in Hooded Lister rats by direct intrahepatic injection of 10^6 HSN sarcoma cells derived from cell culture.This produced two discrete tumour deposits with a median percentage hepatic replacement of 15.6 %.

(6) Abnormalities in the hepatic blood flow pattern were detected in animals with liver tumour.

(7) The HSN tumour model produces deposits that are hypovascular relative to the surrounding liver parenchyma.

(8) Perfusion with blood from a tumour bearing animal results in a reduction in flow through an isolated small segment due to an increase in vascular resistance.

(9) This increase in resistance was abolished by perfusion with blood from a control animal.

(10) Both tumour within and outwith the liver result in a reduction in splanchnic blood flow.

(11) Tumour outwith the liver results in a reduction in blood flow to both the liver and splanchnic vascular bed.

(12) Hepatic arterial blood flow does not change with the development of tumour within the liver.

(13) The calculated HPI was similar in animals with tumour in the flank compared to controls.

(14) The calculated HPI was significantly altered in animals with intrahepatic tumour compared to animals with flank tumours and controls.

TREATMENT OF METASTASES:

Section 1 - First Order Targeting:

(1)Guaric acid polymer:

(15) There was minimal demonstrable targeting of 5-FU to the liver using the gauric acid polymer. The pharmacokinetic profiles of the polymer and free drug were similar,suggesting rapid breakdown of the polymer/label complex.

(2)HPMA polymer:

(16) Using the HPMA polymer,60% of the recovered dose was present in the liver at 5 minutes,increasing slightly at 10 and 30 minutes.

(17) Less than 1% of the HPMA polymer targeted to tumour tissue following i.v administration, with a tumour to liver ratio of 0.07:1.

(18) Regional administration had little effect on the distribution of the HPMA polymer, except that tumour tissue concentration was higher following intra-arterial administration. The tumour to liver ratio was still only 0.12:1

(3)Haematoporphyrin derivative:

(19) Half of the administered haematoporphrin derivative remained 11 days following injection,the remainder having been excreted.

(20) The HpD was concentrated in the liver,spleen,kidney and tumour.The tumour to liver ratio was maximal on day 1 (0.75:1). There is a gradual relative concentration of HpD in the liver and spleen due to its metabolism via the reticuloendothelial system.

Section 2 - Second order targeting:

(21) Mean arterial blood pressure was significantly raised following the injection of angiotensin II.

(22) The intra-arterial injection of DSM significantly increased the retention of marker in both liver and tumour tissue one minute after injection. Similarly, the intra-arterial injection of angiotensin II plus DSM significantly increased the retention of marker in both liver and tumour tissue one minute after injection.

(23) The tumour to liver ratio was unaltered one minute following the injection of either DSM alone or angiotensin II plus DSM.(0.7:1)

(24) The intra-arterial injection of DSM significantly increased the retention of marker in tumour tissue 90 minutes after injection, whereas the retention of marker in the surrounding liver had returned to control values due to portal washout of marker. The intra-arterial injection of angiotensin II plus DSM significantly increased the retention of marker in tumour tissue 90 minutes after injection. At this time point the retention of marker in the surrounding liver had returned to control values.

(25) The tumour : liver ratio was significantly raised 90 minutes following the injection of DSM alone(2.09:1).

(26) The tumour : liver ratio was significantly raised 90 minutes following the injection of angiotensin II plus DSM.(5.22:1)

(27) The administration of angiotensin II plus DSM results in a significantly better tumour : liver ratio than DSM alone.

CHAPTER 4

DISCUSSION

SECTION 1

IMAGING OF HEPATIC METASTATIC DISEASE:

Until recently the early detection of liver metastases arising from primary colorectal carcinoma was more of academic interest than of clinical importance to the patient. The recent confirmation that the subsequent survival following surgical treatment of the colorectal primary may be influenced by chemotherapy or surgery,particularly as these treatments are associated with significant morbidity and the benefit appears to be stage specific,has placed increased significance on the accurate staging of patients suffering from colorectal carcinoma.

Improvements in technology have rendered many of the early studies on the accuracy of imaging techniques obsolete,as sensitivities quoted may be surpassed using modern equipment. In addition the simple detection of metastases is no longer sufficient for treatment planning.Evaluation of number,size and location are essential if resection is considered. Consequently the pattern of investigations chosen may differ from patient to patient.

Since the early 80's computed tomography has been the preferred screening test for the detection of metastases.Advances in technology and technique,using fast scanning sequences and contrast CT angiography, have resulted in a sensitivity of up to 90%. Problems arise with single small metastases and metastases in patients with synchronous hepatic parenchymal disease. In these cases,lesions may be missed or additional investigations required to clarify the diagnosis. Therefore density dependent imaging techniques are insufficiently sensitive to identify a target population for effective chemotherapy.

Previous studies of chemotherapy have shown that although improvements in survival can be achieved in patients with established metastatic disease using either systemic or regional chemotherapy,the treatment is primarily palliative,the only potential for cure in patients with metastases being surgical resection. This,however,is a feasible option in less than 5% of these patients.

Adjuvant therapy on the other hand has been associated with a reduced incidence of overt metachronous metastases and increased long term survival presumably by treating the patients at a micrometastatic stage when the tumour load is small. Of those patients undergoing an apparently curative resection, only 40-45% go on to develop metachronous metastases. Consequently if applied indescriminantly,adjuvant therapy,with its considerable morbidity, would be administered unnecessarily to over half of these patients.

This highlights the potential importance of the hepatic perfusion index in the management of patients with colorectal carcinoma. Not only is the sensitivity in excess of 90% in the detection of established metastases,the separation of patients undergoing apparently curative surgery into those at low or high risk of developing metachronous hepatic metastases would allow some selectivity regarding the administration of adjuvant chemotherapy.

The results of our present studies have confirmed that by the adoption of the high administered activity technique assessed in this thesis,several of the previously reported problems associated with the dynamic scintigraphy may be avoided. Whereas with the former technique up to 14%(Tindale et al,1987) of studies were unsuitable for analysis,we have found only one study impossible to process due to complete overlap of the lung and liver regions of interest.

Similarly the reproducibility of the analysis of studies is improved using the high administered activity technique(Goldberg et al,1987), both on serial analysis or on analysis by two observers. Whereas the difference between the two techniques is not great,the reduction in variability may be of importance in improving accuracy, particularly in studies close to threshold value. It is possible that the greater statistical uncertainty associated with the lower administered activities, may account for some of the conflicting results in previous studies of the diagnostic and prognostic value of the hepatic perfusion index.

Although the sensitivity in the presence of proven metastases is excellent the specificity is poor due to the large number of apparently false positive results. Leveson,in 1985,demonstrated that 94% of patients with metastases at the time of surgery have an abnormal HPI,and that over 60% of those patients who had an abnormal HPI but no demonstrable evidence of disseminated disease on presentation, had developed metastases within one year. Consequently the specificity increases with time, an abnormal HPI identifying a subgroup that may have the most to gain from adjuvant therapy. This prognostic value of the HPI, initially reported by Leveson in 1985,has yet to be reproduced by other centres,and we are consequently following these patients to determine their outcome.

BLOOD FLOW CHANGES ASSOCIATED WITH THE HEPATIC PERFUSION INDEX.

For over 10 years it has been known that abnormalities in liver perfusion occur with the development of metastases,detectable by dynamic hepatic scintigraphy(Parkin et al,1983;Leveson et al,1985;Sarper et al,1981). Because of the difficulties in directly measuring organ blood flow in humans,animal models of hepatic colorectal metastases have been used to investigate changes in the blood flow pattern. Previous studies by Nott(Nott et al,1989) and Hemingway(Hemingway et al,1991) have demonstrated that the HPI is abnormal during both the micro-metastatic and macroscopic stages of tumour development in both hypervascular and hypovascular rodent colorectal tumour models,the magnitude of that abnormality increasing with the growth of the tumour. Because of the progressive nature of the blood flow changes,the HPI may be within the normal range at the earliest stage of the development of slowly growing tumours(Cooke et al,1987;Hemingway et al,1991).

Whether patients that have an initially normal HPI,but develop metastases,do so because their intrahepatic disease is minimally advanced or seeded by cells shed at laparotomy,a repeat HPI at a later time may reveal abnormalities that would predict the subsequent development of overt liver metastases. Unfortunately due to the high administered activity we at present have not obtained ARSAC(Administration of Radioactive Substances Advisory Committee) approval for sequential studies. If the predictive value of the measurements is confirmed, temporal monitoring of the HPI may be possible in the future.

There has been some controversy as to why the blood flow pattern to the liver altered in the presence of metastases. It has been known for over 30 years that the blood supply to metastases was principally derived from the hepatic artery(Breedis and Young,1954;Ackerman et al,1969;Lien and Ackerman,1970; Ackerman,1972). These anatomical studies were not quantitative,and the increase in the proportionate contribution of the hepatic artery to total liver blood flow has been assumed to be as a result of increased hepatic arterial demand(Boyd et al,1978; Leveson et al,1985;Wraight et al,1982).

Using a combination of dynamic scintigraphic and reference microsphere techniques,Nott demonstrated in the hypervascular Walker cell model of colorectal metastases, that the abnormality detected in the HPI was a result of reduced portal venous inflow and not an increase in hepatic arterial flow, which remained constant(Nott et al,1989). These findings were confirmed by Hemingway using the HSN sarcoma cell model,who suggested that the alteration in hepatic proportionate flow was due to an increase in the splanchnic vascular resistance,with a consequent reduction in portal venous flow(Hemingway et al,1991).

Whereas Nott had demonstrated the development of arterio-systemic shunting in the Walker model, Hemingway confirmed that the HPI was also abnormal in a non-shunting model. This disproved the initial theory that the reduction in portal venous flow was due to increased intrahepatic resistance secondary to the development of arterio-venous shunting. Furthermore, using the HSN model Hemingway was able to demonstrate that the changes occurred principally due to an increase in the splanchnic vascular resistance,resulting in a reduction in portal venous flow with only a small increase in intra-hepatic portal vascular resistance.

Although the isolated small bowel cross perfusion experiment developed in this thesis is subject to many experimental pitfalls, with care and practice a stable model within physiological limits can be maintained. The homeostasis of intestinal perfusion is complex with more than one factor acting simultaneously. We observed a trend toward reduced perfusion with time presumably due to progressive metabolic disturbance. For this reason the order of perfusion by tumour bearing and control animals was randomised,and only the first pair of measurements was used for formal comparison, Changes in flow could be reproduced quantitatively for several subsequent measurement pairs in the majority of experiments. Similarly there was a significant range of flows obtained through different segments, as both control and tumour bearing animals perfused the same segment,the intersegmental variation should not effect the final result.

There was a significant trend towards greater intestinal blood flow during periods of perfusion by control animals with increased calculated vascular resistance during perfusion by tumour bearing animals. This provides strong evidence that there is a circulating agent in the blood of tumour bearing animals which results in splanchnic vasoconstriction.

If the tumour is producing a vasoconstricting agent,why does the hepatic perfusion index only become abnormal on the development of intrahepatic tumour,and not with the primary lesion? The results of the reference microsphere experiment,looking at the effect of intrahepatic and intrahepatic tumour may help to explain this phenomenon.

Calculated splanchnic blood flow fell in the presence of both intrahepatic and extrahepatic tumour relative to controls. Hepatic arterial flow fell in the presence of extrahepatic tumour but not with tumour within the liver. The contribution of the hepatic arterial flow in animals with intrahepatic tumour therefore increased in

relative terms,leading to a raised hepatic perfusion index which concurs with the earlier findings of Nott and Hemingway (that the hepatic arterial flow remains constant with the development of intrahepatic tumour).

Taken together the findings of the cross-perfusion study and the reference microsphere study would suggest that both extra-hepatic and intrahepatic tumours may produce a vasoconstricting agent,but in the case of intrahepatic tumour there is local preservation of arterial flow.

SECTION II

THE TREATMENT OF HEPATIC METASTASES

Surgery is the primary treatment modality for patients with colorectal cancer. The majority of colorectal tumours are "operable",nearly 50% of patients develop recurrent disease,even if all the tumour is resected. Over the last 20 years,research has concentrated on chemotherapeutic prevention or treatment of tumour recurrence or metastases,with only minor improvements in survival. This potential benefit is small in relation to inter-surgeon variations in long term results and survival(McArdle and Hole, 1991). It is important to remember therefore the importance of adequate primary surgery in any discussion of the management of colorectal carcinoma.

Even in the most skilled hands, the recurrence rate in patients with colorectal cancer is high, the most common,and often only,site of overt disease at death being the liver. Several studies have demonstrated that chemotherapeutic regimes containing 5-FU provide significant improvements in survival whether given in an adjuvant setting(Windle et al,1985;Moertel et al,1990;Laurie et al,1989) or in the presence of established metastatic disease(Poon et al,1989;Erlichman,1988). These treatments result in significant side effects in up to 30% of patients,some of which may be life- threatening.

The goal of those involved in surgical oncology has long been to develop treatments that would recognise and selectively kill tumour cells. Widder in 1979,formalised the concept of targeting; to the organ,the tumour and the abnormal cells within that tumour. Tertiary targeting remains an area of experimental research,but first and second order targeting have been used in clinical practice to improve response rates and reduce systemic toxicity.

First order targeting:

Organ specific administration of chemotherapy has traditionally been achieved by surgical means. The choice of the chosen route of administration(portal or hepatic arterial) is dependent on whether the treatment is to be given in an adjuvant setting or in the presence of established metastases. Both portal venous and hepatic arterial catheterisation can result in significant surgical complications in addition to the morbidity associated with the chemotherapeutic regime, and therefore non-surgical means of achieving organ specific delivery provides an attractive alternative.

Galactose-terminating neoglycoproteins and glycoproteins (Duncan et al,1983) accumulate in hepatocytes *in-vitro* and *in-vivo* due to a specific receptor mediated pinocytotic uptake mechanism (Ashwell 1982). The incorporation of chemotherapeutic agents with these carrier molecules will result in an alteration in the body distribution profile of a drug,resulting in concentration of that drug within the liver. In this thesis I have evaluated the targeting potential of two such galactose terminating polymers,in an animal model of colorectal metastases, to assess both organ and tumour selectivity.

The 5-FUAA/guaric acid polymer developed in the Liverpool University Department of Bioengineering,was a linear manose chain of molecular weight approximately 220,000 daltons. The radio-labelled portion ,the 5-FU, was covalently bonded via the primary carbon of an acetyl linkage. Intrahepatic targeting was disappointing, levels in liver and tumour being only slightly greater than that following the administration of free 5-FU.

The blood pharmacokinetic profile following the systemic administration of the polymer was only slightly different than that following administration of free drug with a similar t_{max} (4 mins) and a prolonged $t_{1/2}$ of approximately 7.5 minutes,. The AUC_{5FU} : $AUC_{polymer}$ ratio of 1:1.3 suggest slightly greater total drug exposure following polymer exposure which could explain the minimally increased levels in liver rather than any organ specific targeting.

In addition there was also rapid excretion of radioactivity into the urine,inconsistent with continued attachment of the labeled 5-FU to a polymer molecule of that size (220,000 daltons). This suggests that although the stability of the 5-FU/guarin conjugate had been confirmed *in-vitro*,the covalent acetyl linkage may be quickly degraded *in-vivo* releasing free radiolabelled 5-FU,for systemic distribution. Because following disaggregation of the 5-FU/polymer conjugate the galactose/ manose backbone is unlabelled,there may well have been unrecognised intrahepatic accumulation. Further work is required to develop methods of labelling the backbone itself,initially for distribution studies,and, if localisation does occur, an alternative mechanism of linkage of the active drug to delay release until intrahepatic localisation has occurred.

By contrast the Keele University polymer,N-(2-hydroxypropyl) methacrylamide, has been developed to a much greater degree. Although originally developed as a plasma expander(Kopecek et al,1973) a programme of research into potential hepatic selectivity *in-vitro* and in mice has resulted in the development of a drug carrier system suitable for hepatic targeting. Variables such as galactose content and side chain composition have been investigated to maximise delivery.

The HPMA polymers have oligopeptide linkages that are stable in the circulation(Rejmanova et al,1985) but degradable (releasing free drug) intracellularly following pinocytotic inclusion by lysosomal thiol dependent (cysteine) proteases(Duncan et al,1983;Rejamanova et al,1983). The aim of these studies was to evaluate the HPMA polymer in an animal model of colorectal metastases,and in addition to study the effect of regional delivery.

The serum levels following injection of the HPMA polymer follows a first order pharmacokinetic pattern,with a t_{max} of less than one minute and a $t_{1/2}$ of 3 minutes.Interestingly this pattern was unaltered by regional administration,indicating that the hepatic extraction capacity is easily overwhelmed,further extraction occurring on recirculation. The total drug exposure following regional administration, as assessed by the AUC ratio, was minimally lower following regional administration which may indicate a minor degree of first pass uptake.

The distribution profile of the HPMA polymer shows excellent localisation with the liver,with up to 60% of the administered dose being present in the liver within 5 minutes.As expected there would appear to be a small benefit in the amount of polymer in tumour tissue when given by the intraarterial route over both systemic and intraportal administration. The overwhelming localisation within the normal hepatic parenchyma results in tumour levels a tenth of those of surrounding tissue,despite regional administration. This carrier molecule has therefore little to offer in the treatment of established metastases,but this may possibly have a role in the management of micrometastatic disease.

Whereas both the 5-FU/guarin and HPMA polymers were attempts at developing carrier systems for active drugs,the radiolabelled haematoporphyrin derivative was used as a potentially active component in its own right. When exposed to electromagnetic radiation of a suitable wavelength, the electrons of the

photosensitiser molecule are stimulated into an "excited state" -the electrons having moved into an unstable higher energy plane. On decay, these electrons release their potential energy,forming cytotoxic radicals,the most important of which is singlet oxygen. Electromagnetic radiation within the visible spectrum (400-800nm) is most commonly used,but in theory radiation at other wavelengths has the potential for excitation.

Photodynamic therapy is established in the treatment of many superficial tumours. Two principle problems limit the application of photodynamic therapy in solid organ tumours such as hepatic metastases: firstly access difficulties and secondly the limitations posed by the poor penetration of visible light through solid tissue. By using radiation of much longer wavelength,such as x-rays,penetration and access cease to provide difficulties. These preliminary studies are being paralleled by *in-vitro* studies of xray sensitisation of HpD by T.Wheldon,Beatson Institute,Glasgow.

The first reported attempts at photodynamic therapy employed eosin and fluorescein to treat skin cancers in 1906 The natural affinity of porphyrins for tumour tissue was first noticed in 1924 when Policard observed red fluorescence from experimental animal sarcomas exposed to light,due to the accumulation of endogenous porphyrins within the tumour tissue. Auler in 1943 injected porphyrins into tumour bearing animals and confirmed subsequent fluorescence(Moan,1986). Biochemical manipulation of the composition of the injected porphyrin can further improve the tumour localising properties(Lipson et al,1961;Dougherty et al,1984)

This affinity for tumour tissue provides positive tumour to host tissue ratios for most tumour sites in the body. Because of its inaccessibility for photodynamic therapy,the distribution for tumour within the liver was largely unknown. The natural metabolism of porphyrins results in the breakdown of the porphyrin ring in the

reticuloendothelial system,and subsequent transhepatic excretion. The results from these experiments have shown that excellent tumour levels are achieved compared to most organs, but the reticuloendothelial localisation prevents the development of positive tumour to liver parenchymal ratios following administration.

There are two potential solutions to this problem: firstly to employ a photosensitiser that is not excreted by the hepatic route and secondly to further enhance the localisation within liver tumour. Unfortunately at present nearly all available photosensitisers are large molecules,loosely based on the porphyrin ring structure, undergoing hepatic breakdown. Prof. S Brown from Leeds,is at present developing photosensitisers from derivatives of methylene blue,whose size would potentially allow renal excretion (Personal communication). These are still experimental molecules and have not yet been evaluated adequately *in-vitro*,their affinity for tumour tissue being unknown.

An alternative strategy is to improve tumour localisation of the photosensitiser by using monoclonal antibodies linked to the photosensitiser. Dr S.Eccles of the Royal Marsden Hospital,has developed monoclonal antibodies to the HSN tumour cell line and this forms the basis of prospective future work.

Comparing the localisation of the HPMA molecule with that of the porphyrins indicates that both provide reasonable targeting to hepatic parenchymal tissue,but only porphyrins achieve significant tumour levels. The reliance of the HPMA molecule on the presence of the hepatocyte galactose receptor limits its potential clinical use in that the carrier targets normal cells in preference to tumour tissue.

Second Order Targeting:

Despite accumulating clinical experience in the manipulation of hepatic blood flow to optimise the regional delivery to tumour tissue the rationale for using degradable emboli or vasoactive agents for regional targeting has not been fully evaluated at an experimental level.

Tumour vessels are immature and lack the smooth muscle elements to react with vasoconstrictor agents(Mattson et al,1977;Mattson et al,1978). Any response following the administration of a vasoconstricting agent therefore occurs predominately in the surrounding normal hepatic parenchyma. This results in temporary relative tumour hypervascularity with potentially improved regional delivery of drug to tumour tissue. We have previously demonstrated that vasoactive agents,such as angiotensin II or phenylephrine,can improve the delivery of a regionally administered drug to tumour tissue(Hemingway et al,1991).

In addition,it has been shown that in both animals(Fujimoto et al,1985;Sigurdson et al,1986;Flowerdew et al,1987;Nott et al,1989) and in man(Dakhil et al,1982;Gyves et al,1983;Mavor et al,1987; Starkhammer et al,1987;Goldberg et al,1988;Lorenz et al,1989;Hunt et al,1990) regionally administered degradable starch microspheres reduce the hepatic arterial flow with improved retention of drugs or markers within tumour tissue. The aim of this study was to determine whether the combination of vasoactive agents with degradable microspheres could further enhance the delivery of a regionally delivered marker to tumour tissue.

The administration of DSM results in temporary hepatic arterial stasis. The arterial vascular diversion as a result of the simultaneous or subsequent administration of angiotensin II will be negated if there is little or no flow along the vessel,thus explaining why the administration of angiotensin II prior to the DSM and marker provided the best results in the pilot experiments.

This study confirmed that DSM alone can significantly increase the retention of marker in tumour with a twelve fold increase over controls at one minute. In contrast to previous studies(Cooke and Chang,1990) we have shown no immediate preferential delivery to tumour tissue. It has been previously suggested that the increased retention in tumour tissue was as a result of a redistribution of intrahepatic blood flow from hepatic parenchyma towards tumour tissue. The results of this study would suggest that the DSM is distributed according to blood flow,which initially results in retention of marker in both tumour and liver tissue at one minute without altering the tumour to liver ratio. By 90 minutes, we have demonstrated relatively greater retention in tumour tissue,with almost complete washout from normal liver tissue ,probably as a result of continued portal flow to normal tissue.

Previous studies in our group have suggested that angiotensin II results in a redistribution of intrahepatic blood flow towards tumour tissue(Hemingway et al,1991). The prior administration of angiotensin II would therefore be expected to result in preferential delivery of the DSM and marker to the tumour tissue. Whilst we were unable to demonstrate an immediate advantage of combination targeting over DSM alone,by 90 minutes there was significantly greater retention of marker in tumour tissue compared to DSM alone,and a twelve fold increase over control animals. Moreover the tumour to liver ratio of retained marker was significantly improved using combined angiotensin II and DSM over DSM alone,with a concentration in tumour tissue five times that of surrounding liver parenchyma.The

improved retention of marker in tumour may reflect prolonged blood flow stasis and lack of washout due to initial targeting of the DSM to tumour tissue.

The results of this study would suggest that the combination of angiotensin II and DSM can further improve the delivery of drug to intrahepatic tumour,whilst minimising the exposure of normal hepatic parenchyma to potentially hepatotoxic drugs.

CHAPTER 5

CONCLUSIONS

AND PROPOSED RESEARCH

CONCLUSIONS

(1) The measurement of the Hepatic Perfusion Index using a high administered activity,is reproducible both between different observers and on reprocessing studies by a single observer.

(2) The HPI has a diagnostic sensitivity equal to that of computerised tomography.Intraoperative ultrasonography provides little additional information in the early detection of colorectal metastases over standard techniques. Rather than detection its principle role would appear to lie in the delineation of the intrahepatic vascular anatomy in the small number of patients in whom resection is being considered.

(3) The specificity of the HPI was poor,however if as suggested, those patients who are apparently disease free but with a raised HPI develop metachronous disease the specificity of the HPI will improve and the sensitivity and specificity of all other methods of detection will reduce.

(4) The HSN sarcoma cell model is a reliable model of colorectal metastases,producing tumours that are both slow growing and hypovascular,as in many human colorectal metastases.

(5) The change in the hepatic and splanchnic haemodynamics with the development of intrahepatic tumour in the HSN model,is due to a circulating vasoactive agent increasing splanchnic vascular resistance.

(6)Both extra-hepatic and intra-hepatic tumours are associated with reduced splanchnic vascular inflow.

(7)The development of an abnormal HPI in animals with intravascular tumour is due to local preservation of hepatic arterial inflow despite splanchnic vasoconstriction.

(8) There is minimal hepatic targeting of 5-FU following injection of a 5-FU guarin/galactose polymer. This may be due to dissociation of the 5-FU from the polymer backbone.

(9)Excellent targeting to hepatic parenchyma is possible using a HPMA polymer backbone,but intrahepatic tumour levels are minimal.Regional delivery has little effect on polymer distribution. Small increases in tumour levels are possible using an intraarterial approach.

(10) Similar levels of hepatic targeting are possible with using haematoporphyrin derivatives,with improved tumour localisation. Despite this tumour: liver ratios remain less than 1.

(11) Second order targeting of a chemotherapeutic marker using either degradable microspheres or vasoactive agents may be further improved by a combination approach. Administration of the vasoactive agent prior to the emboli provides the best results,producing a tumour:liver ratio in excess of 5:1 at 90 minutes.

FUTURE WORK

Although the work contained in this thesis has been successful in providing solutions for many of the questions for which the studies were designed,the work has generated more questions than answers. Many of the studies were in collaboration with other Departments,and fortunately these links are continuing,and a summary of the immediate projects to be carried out in our own Department and in other units is presented below:

(1) The reported ability of the HPI to predict the development of metachronous metastases in patients thought to be free of metastases should be prospectively evaluated.

(2) The role of the HPI in the investigation and management of patients with tumours other than colorectal primaries should be assessed.

(3) The combination of angiotensin II and DSM in second order targeting of regional chemotherapy requires evaluation in a phase one clinical study.

In conjunction with the University Department of Physiology:

(4) An attempt to identify the vasoactive agent responsible for the change in splanchnic vascular resistance is required,initially studying the response of vascular smooth muscle to tumour bearing plasma with sequential selective blockade in an *in-vitro* muscle ring organ bath experiment.

(5) The effect of tumour bearing plasma on the resistance vessels(Arterioles 100-150μm diameter) of the splanchnic vascular bed,should be studied using an *in-vitro* isolated perfusion experiment and a *in-vitro* "Myograph" preparation.

In conjunction with the University of Liverpool:

(6) The stability of the 5-FU guarin/galactose polymer in rat plasma should be assessed, and if as suspected there is rapid breakdown of the conjugate, further distribution studies may be performed with an improved preparation.

In conjunction with the University of Keele:

(7) The poor tumour levels achieved using the HPMA polymer do not undermine its potential for treating patients with micrometastatic disease. The effect of the excellent hepatic parenchymal targeting should be assessed a phase 1 clinical study in patients with colorectal carcinoma.

In conjunction with the Beatson Oncology Centre,Glasgow,and the Royal Marsdon Hospital,London.

(8) Alternative methods of improving tumour:liver ratios of the haematoporphyrin derivatives are required. The use of monoclonal antibodies to improve intratumour localisation should be assessed. The effect of using a photosensitiser of low molecular weight,allowing renal excretion may also prevent intrahepatic accumulation of the photosensitiser. These experiments are being paralleled by *in-vitro* work investigating stimulation of photo-sensitisers by non ionising radiation other than visible light.

CHAPTER 6

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CHAPTER 7

APPENDICES

SECTION I

RESULTS OF CLINICAL STUDIES

APPENDIX 1

CLINICAL STUDY on the REPRODUCIBILITY of the HPI

Patient number	HPI values (first observer)				(second observer)	inter-observer difference
Liver metastases:						
1	0.89	0.90	0.89	0.90	0.91	+0.02
2	0.58	0.53	0.54	0.51	0.59	+0.01
3	0.44	0.48	0.45	0.46	0.51	+0.07
4	0.43	0.43	0.43	0.41	0.47	+0.04
5	0.39	0.39	0.36	0.33	0.38	-0.01
6	0.52	0.53	0.55	0.55	0.52	0.00
No Liver metastases:						
7	0.33	0.37	0.34	0.34	0.39	+0.06
8	0.35	0.35	0.37	0.36	0.36	+0.01
9	0.40	0.41	0.36	0.36	0.38	-0.02
10	0.12	0.08	0.14	0.18	0.15	+0.03
11	0.19	0.21	0.21	0.18	0.27	+0.09
12	0.37	0.34	0.37	0.37	0.40	+0.03
13	0.33	0.33	0.34	0.34	0.40	+0.07
14	0.26	0.21	0.25	0.32	0.33	+0.07
15	0.54	0.59	0.60	0.50	0.50	-0.04
16	0.46	0.48	0.54	0.50	0.57	+0.11
17	0.22	0.26	0.26	0.20	0.30	+0.08
18	0.25	0.27	0.27	0.28	0.30	+0.05

APPENDIX 2

PROSPECTIVE IMAGING RESULTS

Patient No.	U/S	CT	HPI	Static Palpn	I.O.U/S	CONSENSUS
1	N	N	36	N	N	N	-
2	N	N	38	N	N	N	-
3	N	N	15	N	N	N	-
4	N	N	20	N	N	N	-
5	N	N	64	N	N	N	-
6	POS	POS	43	POS	POS	POS	+
7	POS	POS	38	N	N	N	+
8	POS	POS	54	POS	POS	POS	+
9	N	N	54	POS	N	N	-
10	N	N	49	N	N	N	-
11	N	N	27	N	N	N	-
12	N	N	31	N	N	N	-
13	N	N	42	POS	N	N	-
14	POS	POS	59	POS	POS	POS	+
15	N	N	46	POS	POS	N	+
16	POS	POS	29	POS	POS	POS	+
17	N	N	43	N	N	N	-
18	N	POS	41	N	N	N	+
19	POS	POS	52	POS	POS	POS	+
20	N	N	focal POS		N	N	-
21	N	N	74	POS	N	N	-
22	POS	POS	52	POS	POS	POS	+
23	POS	POS	55	POS	POS	POS	+
24	N	N	12	N	N	N	-
25	N	POS	47	POS	POS	POS	+
26	N	N	40	N	N	N	-
27	POS	POS	56	POS	POS	POS	+
28	N	POS	39	POS	N	N	+
29	POS	POS	57	POS	POS	POS	+
30	N	N	62	N	N	N	-
31	N	N	39	N	N	N	-
32	N	N	31	N	N	N	-
33	N	N	30	N	N	N	-
34	N	N	57	N	N	N	-
35	POS	POS	55	POS	POS	POS	+
36	N	POS	67	N	N	N	-
37	N	POS	46	N	N	N	-
38	POS	POS	39	POS	POS	POS	+
39	POS	POS	38	POS	POS	POS	+
40	N	N	50	N	N	N	-

PATIENTS IN WHOM ALL INVESTIGATIONS WERE NOT COMPLETED:

Patient No.	U/S	CT	HPI	Static	Palpn	I.O.U/S	CONSENSUS
1	N	N	47	N	-	-	-
2	POS	POS	51	POS	-	-	+
3	N	N	34	POS	N	-	-
4	POS	POS	89	POS	POS	-	+
5	POS	POS	54	POS	POS	-	+
6	N	N	46	POS	N	-	-
7	N	N	37	N	-	-	-
8	N	N	34	N	N	-	-
9	N	N	26	N	N	-	-
10	N	N	26	N	N	-	-
11	POS	POS	72	POS	-	-	+
12	N	POS	50	N	-	-	+
13	N	POS	27	N	N	-	-
14	POS	POS	79	N	POS	-	+
15	POS	POS	72	POS	-	-	+
16	POS	POS	82	POS	POS	-	+
17	POS	POS	53	N	-	-	+
18	POS	POS	79	POS	-	-	+
19	N	N	49	N	-	-	-
20	N	N	44	N	-	-	-
21	N	POS	40	N	-	-	+
22	N	N	54	POS	-	-	-
23	N	POS	40	POS	-	-	+
24	N	N	57	N	-	-	-
25	N	N	22	N	-	-	-
26	N	N	27	N	-	-	-
27	POS	POS	63	POS	-	-	+
28	POS	POS	43	POS	-	-	+
29	POS	POS	94	POS	-	-	+
30	N	N	54	POS	POS	-	+
31	POS	POS	57	POS	POS	-	+
32	POS	POS	48	POS	POS	-	+
33	N	POS	48	N	POS	-	+

SECTION 2

RESULTS OF

EXPERIMENTS EXAMINING THE

HAEMODYNAMIC CHANGES THAT OCCUR

WITH THE DEVELOPMENT OF

INTRAHEPATIC AND EXTRAHEPATIC

TUMOUR

APPENDIX 3

Cross perfusion 1

Segment number	Tumour			Control			Diff.in mean resistance
	Press	Flow	Resist	Press	Flow	Resist	
1	100	1.4	71.4	105	1.8	58.3	
	100	1.2	83.3	100	1.7	52.6	31.3
	100	1.0	100	100	2.0	50	
2	85	1.9	44.7	100	2.2	45.4	
	90	2.0	45.0	105	2.0	52.5	-2.7
	95	2.4	39.6	95	2.4	39.6	
3	95	1.4	67.8	110	1.0	110	
	90	1.6	56.2	110	1.0	110	-53.2
	95	1.4	67.8	105	0.8	131.2	
4	95	2.8	33.9	105	2.8	33.9	
	95	1.8	31.2	105	3.6	27.8	7.1
	95	2.0	35	105	3.2	32.8	
5	95	2.8	33.9	105	3.4	30.9	
	100	3.2	31.2	105	3.6	29.2	3.6
	105	3.0	35	105	3.6	29.2	
6	100	3.8	26.3	100	3.8	26.3	
	95	3.4	28.2	95	3.8	25.3	1.4
	100	3.8	26.3	105	4.2	25.0	
7	100	1.9	52.6	90	2.6	34.6	
	90	2.1	42.8	85	2.4	35.4	12.7
	100	1.9	52.6	90	2.6	34.6	
8	115	1.2	95.8	90	1.3	69.2	
	115	1.2	95.8	95	1.2	79.2	26.6
	110	1.1	100	95	1.5	63.3	
9	100	0.4	250	105	1.6	60.0	
	100	0.4	255	110	1.8	60.0	172.1
	100	0.5	200	110	1.6	68.7	
10	95	0.4	240	110	1.6	68.7	
	100	0.4	245	105	2.0	53	186.1
	100	0.4	245	110	2.4	45.8	
11	125	0.8	156.3	100	1.5	66.7	
	120	1.2	100	100	1.8	55.6	56.5
	120	1.2	100	110	1.7	64.7	
12	100	1.8	55.6	100	3.4	29.2	
	110	1.7	64.7	105	3.2	32.8	35.4
	100	1.6	76.9	100	3.5	28.6	

Cross perfusion 2

Segment number	Tumour			Control			Diff.in mean resistance
	Press	Flow	Resist	Press	Flow	Resist	
1	100	1.2	83.3	100	2.0	50	
	100	1.2	83.3	100	2.4	41.6	36.1
	95	1.3	73.1	100	2.5	40	
2	110	2.1	52.3	100	1.6	62.5	
	100	1.9	52.6	110	2.1	52.3	-8.4
	100	1.8	55.5	85	1.2	70.8	
3	95	1.0	95	110	0.9	122.2	
	100	1.2	83.3	110	1.3	84.6	-4.2
	95	1.2	79.1	100	1.2	83.3	
4	100	1.6	62.5	110	3.3	33.3	
	110	2.1	52.3	100	2.8	35.7	23.5
	100	1.6	62.5	110	2.9	37.9	
5	110	2.4	45.8	100	2.6	38.4	
	100	2.2	45.4	95	2.7	35.1	6.8
	110	2.3	47.8	90	2.0	45	
6		<80		not done			
7		<80		not done			
8	95	0.8	118.7		<80		
	95	0.9	105.5				
	100	0.7	142.8				
9	100	0.4	250	100	0.9	111.1	
	100	0.6	166.6	100	0.9	111.1	111.1
	100	0.4	250	100	0.9	111.1	
10	100	0.8	125	100	1.1	90.9	
	100	0.8	125	90	0.8	112.5	21.9
	95	0.9	105.5	95	1.1	86.3	
11		<80		120	0.4	300	
				115	0.6	191.6	
				100	0.2	500	
12	100	1.2	83.3	100	2.6	38.4	
	110	1.6	68.7	100	2.4	41.6	29.1
	110	1.7	64.7	110	2.2	50	

APPENDIX 4

Results of Reference Microsphere experiments:

Number 1 Tumour - none Reference counts(1ml) = 3000

	weight	counts	Blood Flow	Flow/g
Liver	6.0	2467	0.82	0.13
Tumour				
Kidney 1	0.8	12254	4.08	5.1
Kidney 2	0.8	11220	3.74	4.67
Spleen	0.5	1118	0.37	0.74
Heart	0.7	6848	2.28	3.25
Lung	1.0	2097	0.69	0.69
Stomach	1.7	1937	0.64	0.37
Pancreas	0.9	1061	0.35	0.38
Sm.Bowel	6.0	26566	8.85	1.48
Colon	3.0	3354	1.12	0.37

Liver Flow	=	0.82	
Splanchnic Flow	=	11.34	
Splanchnic weight	=	12.1	0.94
Calculated HPI	=	0.067	

Number 2 Tumour - none Reference counts(1ml) = 1403

	weight	counts	Blood Flow	Flow/g
Liver	6.8	1451	1.034	0.152
Tumour				
Kidney 1	1.0	4954	3.53	3.53
Kidney 2	0.95	5278	3.76	3.95
Spleen	0.8	849	0.6	0.75
Heart	0.9	3418	2.43	2.7
Lung	1.3	1043	0.74	0.56
Stomach	1.4	1115	0.79	0.56
Pancreas	1.2	1350	0.96	0.80
Sm.Bowel	6.4	12287	8.75	1.36
Colon	3.2	1885	1.34	0.42

Liver Flow	=	1.034	
Splanchnic Flow	=	12.48	
Splanchnic Weight	=	13.0	0.96
Calculated HPI	=	0.076	

Number 3 Tumour - none Reference counts(1ml) = 3624

	weight	counts	Blood Flow	Flow/g
Liver	9.64	3316	0.91	0.094
Tumour				
Kidney 1	1.05	18206	5.02	4.78
Kidney 2	1.00	16724	4.61	4.61
Spleen	0.8	3346	0.92	1.15
Heart	0.95	19737	5.44	5.72
Lung	1.58	2556	0.7	0.44
Stomach	1.74	2934	0.8	0.46
Pancreas	1.46	6963	1.92	1.31
Sm.Bowel	9.2	30538	8.42	0.91
Colon	4.26	4906	1.35	0.316

Liver Flow	=	0.91	
Splanchnic Flow	=	13.42	
Splanchnic Weight	=	17.5	0.76
Calculated HPI	=	0.063	

Number 4 Tumour - none Reference counts(1ml) = 5203

	weight	counts	Blood Flow	Flow/g
Liver	9.4	4789	0.91	0.096
Tumour				
Kidney 1	1.1	25280	4.85	4.40
Kidney 2	1.1	26101	5.01	4.55
Spleen	1.0	5789	1.11	1.11
Heart	0.95	24835	4.77	5.02
Lung	1.45	2577	0.49	0.33
Stomach	1.85	2934	0.56	0.30
Pancreas	1.5	4406	0.84	0.56
Sm.Bowel	9.7	32251	6.19	0.66
Colon	4.7	6195	1.19	0.25

Liver Flow	=	0.91	
Splanchnic Flow	=	9.91	
Splanchnic Weight	=	18.7	0.53
Calculated HPI	=	0.087	

Number 5 Tumour - none Reference counts(1ml) = 1363

	weight	counts	Blood Flow	Flow/g
Liver	6.1	1489	1.09	0.178
Tumour				
Kidney 1	0.85	4912	3.63	4.27
Kidney 2	0.85	5356	3.92	4.64
Spleen	0.7	897	0.65	0.92
Heart	0.8	23860	17.5	21.8
Lung	1.25	1132	0.83	0.66
Stomach	1.7	1160	0.85	0.5
Pancreas	1.1	1387	1.01	0.91
Sm.Bowel	6.4	11771	8.63	1.34
Colon	2.9	1847	1.35	0.46

Liver Flow	=	1.09	
Splanchnic Flow	=	12.51	
Splanchnic Weight	=	12.8	0.98
Calculated HPI	=	0.08	

Number 6 Tumour - none Reference counts(1ml) = 1634

	weight	counts	Blood Flow	Flow/g
Liver	8.4	1217	0.74	0.09
Tumour				
Kidney 1	0.85	5368	3.28	3.85
Kidney 2	0.85	4818	2.94	3.45
Spleen	0.6	482	0.29	0.48
Heart	1.0	4763	2.91	2.91
Lung	1.2	472	0.28	0.23
Stomach	1.5	1861	1.13	0.75
Pancreas	1.15	1425	0.87	0.76
Sm.Bowel	8.4	7566	4.63	0.55
Colon	3.2	2597	1.58	0.49

Liver Flow	=	0.74	
Splanchnic Flow	=	8.52	
Splanchnic Weight	=	14.85	0.57
Calculated HPI	=	0.079	

Number 7 Tumour - flank Reference counts(1ml) = 2260

	weight	counts	Blood Flow	Flow/g
Liver	9.4	1768	0.782	0.08
Tumour	1.9	631	0.13	0.14
Kidney 1	1.0	14440	6.38	6.38
Kidney 2	1.0	12738	5.63	5.63
Spleen	0.7	2497	1.1	1.57
Heart	0.9	8283	3.36	3.73
Lung	1.6	2549	1.12	0.70
Stomach	2.0	2839	1.25	0.63
Pancreas	1.5	2015	0.89	0.59
Sm.Bowel	9.8	13960	6.17	0.62
Colon	4.2	3110	1.37	0.03

Liver Flow	=	0.78	
Splanchnic Flow	=	10.8	
Splanchnic Weight	=	18.2	0.593
Calculated HPI	=	0.068	

Number 8 Tumour - flank Reference counts(1ml) = 2697

	weight	counts	Blood Flow	Flow/g
Liver	9.4	1288	0.48	0.05
Tumour	1.7	1269	0.27	0.27
Kidney 1	1.1	13806	5.11	4.64
Kidney 2	1.1	16636	6.16	5.60
Spleen	0.9	714	0.26	0.29
Heart	0.8	7019	2.6	3.25
Lung	1.4	4943	1.83	1.30
Stomach	1.6	1004	0.37	0.23
Pancreas	1.3	2421	0.90	0.69
Sm.Bowel	9.2	8468	3.13	0.34
Colon	4.6	2988	1.10	0.24

Liver Flow	=	0.48	
Splanchnic Flow	=	5.78	
Splanchnic Weight	=	17.6	0.33
Calculated HPI	=	0.076	

Number 9 Tumour - flank Reference counts(1ml) = 2037

	weight	counts	Blood Flow	Flow/g
Liver	9.4	2926	1.43	0.15
Tumour	2.3	1006	0.19	0.21
Kidney 1	1.0	9519	4.67	4.67
Kidney 2	1.05	10271	5.04	4.80
Spleen	0.7	1353	0.66	0.94
Heart	0.9	8472	4.15	4.61
Lung	1.6	1186	0.58	0.36
Stomach	2.0	2298	1.12	0.56
Pancreas	1.5	3606	1.77	1.18
Sm.Bowel	10.3	16002	7.85	0.76
Colon	4.2	2799	1.37	0.33

Liver Flow	=	1.43	
Splanchnic Flow	=	14.22	
Splanchnic Weight	=	18.7	0.76
Calculated HPI	=	0.10	

Number 10 Tumour - flank Reference counts(1ml) = 2711

	weight	counts	Blood Flow	Flow/g
Liver	9.5	1354	0.50	0.05
Tumour	1.6	1379	0.28	0.31
Kidney 1	1.0	13685	5.04	5.04
Kidney 2	1.1	16184	5.96	5.41
Spleen	1.0	752	0.28	0.28
Heart	1.1	7147	2.63	2.39
Lung	1.6	4975	1.83	1.14
Stomach	1.7	952	0.35	0.21
Pancreas	1.7	2372	0.87	0.51
Sm.Bowel	10.9	8168	3.01	0.28
Colon	4.5	3007	1.10	0.24

Liver Flow	=	0.5	
Splanchnic Flow	=	5.62	
Splanchnic Weight	=	19.5	0.29
Calculated HPI	=	0.08	

Number 11 Tumour - flank Reference counts(1ml) = 2349

	weight	counts	Blood Flow	Flow/g
Liver	10.1	1923	0.82	0.08
Tumour	1.6	474	0.13	0.12
Kidney 1	1.0	14620	6.22	6.22
Kidney 2	0.95	12183	5.18	5.45
Spleen	0.85	2453	1.04	1.22
Heart	0.85	8382	3.56	4.18
Lung	1.4	2619	1.11	0.79
Stomach	1.3	2821	1.2	0.92
Pancreas	1.1	2014	0.85	0.77
Sm.Bowel	9.7	14160	6.02	0.62
Colon	4.1	3023	1.28	0.31

Liver Flow	=	0.82	
Splanchnic Flow	=	10.41	
Splanchnic Weight	=	17.05	0.61
Calculated HPI	=	0.073	

Number 12 Tumour - flank Reference counts(1ml) = 2734

	weight	counts	Blood Flow	Flow/g
Liver	8.1	1288	0.47	0.58
Tumour	1.4	712	0.26	0.19
Kidney 1	1.0	15539	5.68	5.68
Kidney 2	1.05	17175	6.28	5.98
Spleen	0.8	751	0.27	0.34
Heart	0.9	6795	2.48	2.75
Lung	1.4	4766	1.74	1.24
Stomach	1.7	991	0.36	0.21
Pancreas	1.3	2673	0.98	0.75
Sm.Bowel	7.2	7300	2.67	0.37
Colon	3.9	2887	1.05	0.27

Liver Flow	=	0.47	
Splanchnic Flow	=	5.34	
Splanchnic Weight	=	14.9	0.36
Calculated HPI	=	0.08	

Number 13 Tumour - liver Reference counts(1ml) = 7878

	weight	counts	Blood Flow	Flow/g
Liver	9.2	12447	1.58	0.171
Tumour	2.6	1543	0.53	0.075
Kidney 1	1.1	51104	6.48	5.89
Kidney 2	1.0	41898	5.38	5.38
Spleen	1.0	4517	0.56	0.56
Heart	0.9	71218	9.02	10.0
Lung	1.9	3515	0.45	0.24
Stomach	1.4	3218	0.4	0.29
Pancreas	1.7	6049	0.76	0.44
Sm.Bowel	8.2	30803	3.91	0.48
Colon	3.4	9857	1.24	0.36

Tumour/liver ratio	=	0.44	
Liver Flow	=	1.58	
Splanchnic Flow	=	6.87	
Splanchnic Weight	=	15.7	0.44
Calculated HPI	=	0.187	

Number 14 Tumour - liver Reference counts(1ml) = 2086

	weight	counts	Blood Flow	Flow/g
Liver	9.6	2482	1.19	0.12
Tumour	2.3	232	0.28	0.04
Kidney 1	1.2	9427	4.51	3.76
Kidney 2	1.2	9148	4.37	3.53
Spleen	0.9	1459	0.69	0.77
Heart	0.9	7270	3.49	3.87
Lung	1.6	1012	0.49	0.28
Stomach	2.0	364	0.17	0.08
Pancreas	1.8	2888	1.38	0.76
Sm.Bowel	10.3	8824	4.23	0.41
Colon	5.2	2809	1.35	0.25

Tumour/liver ratio	=	0.39	
Liver Flow	=	1.19	
Splanchnic Flow	=	7.82	
Splanchnic Weight	=	20.2	0.39
Calculated HPI	=	0.132	

Number 15 Tumour - liver Reference counts(1ml) = 3263

	weight	counts	Blood Flow	Flow/g
Liver	10.0	3393	1.04	0.10
Tumour	2.1	368	0.35	0.05
Kidney 1	1.2	17857	5.47	4.56
Kidney 2	1.1	14210	4.35	3.95
Spleen	0.9	1025	0.31	0.34
Heart	0.8	11463	3.51	4.39
Lung	1.5	2015	0.61	0.40
Stomach	1.6	1066	0.32	0.19
Pancreas	1.2	3909	1.19	0.95
Sm.Bowel	10.1	15238	4.67	0.46
Colon	4.7	7374	2.26	0.48

Tumour/liver ratio	=	0.55	
Liver Flow	=	1.04	
Splanchnic Flow	=	8.77	
Splanchnic Weight	=	18.5	0.47
Calculated HPI	=	0.106	

Number 16 Tumour - liver Reference counts(1ml) = 2176

	weight	counts	Blood Flow	Flow/g
Liver	7.3	1627	0.75	0.10
Tumour	1.6	600	0.27	0.17
Kidney 1	1.0	9683	4.44	4.44
Kidney 2	1.0	10813	4.96	4.96
Spleen	0.55	1071	0.49	0.89
Heart	0.9	3402	1.56	1.73
Lung	1.3	1408	0.64	0.49
Stomach	1.7	1556	0.71	0.42
Pancreas	1.2	2070	0.95	0.79
Sm.Bowel	7.6	8640	3.97	0.52
Colon	4.2	2691	1.23	0.29

Tumour/liver ratio	=	1.7	
Liver Flow	=	0.75	
Splanchnic Flow	=	7.36	
Splanchnic Weight	=	15.3	0.48
Calculated HPI	=	0.093	

Number 17 Tumour - liver Reference counts(1ml) = 2142

	weight	counts	Blood Flow	Flow/g
Liver	8.1	2377	1.11	0.13
Tumour	1.2	118	0.05	0.04
Kidney 1	1.0	10701	4.99	4.99
Kidney 2	1.0	11678	5.45	5.45
Spleen	0.8	2164	1.01	1.26
Heart	0.9	5989	2.79	3.10
Lung	1.4	633	0.29	0.21
Stomach	1.7	1277	0.59	0.35
Pancreas	1.3	2005	0.93	0.72
Sm.Bowel	7.2	8910	4.16	0.58
Colon	4.1	3092	1.44	0.35

Tumour/Liver ratio	=	0.33	
Liver Flow	=	1.11	
Splanchnic Flow	=	8.14	
Splanchnic Weight	=	15.1	0.54
Calculated HPI	=	0.12	

Number 18 Tumour - liver Reference counts(1ml) = 2396

	weight	counts	Blood Flow	Flow/g
Liver	9.0	2204	0.92	0.10
Tumour	1.8	292	0.06	0.06
Kidney 1	0.9	10784	4.50	5.01
Kidney 2	0.9	11485	4.79	5.32
Spleen	0.8	2151	0.89	1.12
Heart	0.9	6017	2.51	2.79
Lung	1.3	618	0.26	0.19
Stomach	1.7	1277	0.53	0.31
Pancreas	1.3	1954	0.81	0.62
Sm.Bowel	8.3	9976	4.08	0.49
Colon	4.5	3003	1.25	0.28

Tumour/liver ratio	=	0.59	
Liver Flow	=	0.92	
Splanchnic Flow	=	7.56	
Splanchnic Weight	=	16.6	0.45
Calculated HPI	=	0.108	

SECTION 3

RESULTS OF EXPERIMENTS STUDYING METHODS OF FIRST AND SECOND ORDER HEPATIC TARGETING

APPENDIX 5a

5FU ACETATE and 5FU/GUARIC ACID POLYMER DISTRIBUTION STUDIES

Recovery experiment of known amount of 5-FU injected into variable weights of liver parenchyma.

Vial number	Weight (mg)	counts recovered
1	96	240
2	206	4667
3	310	3755
4	398	4852
5	499	4911
6	603	5077
7	0	4684
8	115	4387
9	192	4765
10	320	3989
11	405	5142
12	496	4268
13	587	4643
14	0	4492

5-FU-ACETIC ACID BLOOD RESULTS

Results expressed as a percentage of the injected dose per ml blood

10 minutes

	Animal number					
time	1	2	3	4	5	6
2	0.528	0.628	0.423	0.763	0.391	0.467
4	0.964	1.092	0.863	0.932	0.743	1.156
6	0.436	0.536	0.632	0.396	0.564	0.643
8	0.173	0.289	0.309	0.201	0.276	0.342
10	0.062	0.079	0.127	0.097	0.151	0.119

30 minutes

	Animal number					
time	1	2	3	4	5	6
5	0.643	0.743	0.436	0.546	0.876	0.549
10	0.125	0.097	0.076	0.098	0.135	0.084
15	0.062	0.051	0.042	0.056	0.078	0.042
20	0.056	0.046	0.036	0.042	0.065	0.046
25	0.043	0.048	0.039	0.048	0.067	0.037
30	0.041	0.039	0.038	0.040	0.069	0.036

60 minutes

	Animal number					
time	1	2	3	4	5	6
10	0.097	0.132	0.088	0.118	0.198	0.114
20	0.050	0.063	0.041	0.058	0.103	0.063
30	0.041	0.044	0.032	0.041	0.078	0.052
40	0.039	0.035	0.030	0.039	0.080	0.051
50	0.040	0.040	0.031	0.039	0.076	0.049
60	0.042	0.039	0.034	0.038	0.082	0.052

5-FU/GUARIC ACID POLYMER BLOOD RESULTS

Results expressed as a percentage of the injected dose per ml blood

10 minutes

	Animal number					
time	1	2	3	4	5	6
2	0.678	0.732	0.546	0.698	0.732	0.548
4	1.241	0.981	0.875	1.032	0.971	0.838
6	0.786	0.723	0.675	0.781	0.698	0.447
8	0.564	0.532	0.423	0.518	0.476	0.370
10	0.342	0.431	0.301	0.412	0.340	0.296

30 minutes

	Animal number					
time	1	2	3	4	5	6
5	0.670	0.743	0.562	0.819	0.480	0.651
10	0.406	0.399	0.259	0.488	0.200	0.218
15	0.167	0.122	0.098	0.210	0.087	0.093
20	0.078	0.069	0.051	0.120	0.048	0.057
25	0.056	0.054	0.044	0.111	0.044	0.051
30	0.055	0.048	0.049	0.119	0.047	0.044

60 minutes

	Animal number					
time	1	2	3	4	5	6
10	0.314	0.438	0.378	0.296	0.356	0.444
20	0.081	0.121	0.091	0.078	0.097	0.107
30	0.046	0.055	0.044	0.056	0.054	0.066
40	0.044	0.056	0.049	0.049	0.051	0.041
50	0.049	0.051	0.044	0.049	0.055	0.049
60	0.047	0.055	0.039	0.047	0.046	0.044

5-FU ACETIC ACID DISTRIBUTION PROFILE

Results expressed as a percentage of the injected dose per gram tissue

10 minutes

Liver	Tumour	Kidney	Spleen	Heart	Lung
0.131	0.023	0.314	0.002	0.012	0.006
0.124	0.028	0.179	0.001	0.006	0.015
0.113	0.032	0.270	0.001	0.008	0.010
0.178	0.238	1.554	0.002	0.010	0.048
0.243	0.106	0.958	0.002	0.081	0.012
0.334	0.338	1.069	0.034	0.072	0.156

30 minutes

Liver	Tumour	Kidney	Spleen	Heart	Lung
0.509	0.097	0.970	0.004	0.045	0.071
0.576	0.172	1.798	0.066	0.047	0.088
0.868	0.129	2.689	0.015	0.060	0.075
0.335	0.160	1.757	0.004	0.026	0.048
0.312	0.117	2.056	0.016	0.051	0.030
0.297	0.142	0.438	0.003	0.025	0.004

60 minutes

Liver	Tumour	Kidney	Spleen	Heart	Lung
0.639	0.156	3.042	0.040	0.059	0.095
0.545	0.172	3.410	0.044	0.042	0.024
0.731	0.164	3.415	0.040	0.066	0.063
0.421	0.107	3.478	0.053	0.060	0.056
0.227	0.092	2.992	0.029	0.073	0.060
0.452	0.173	2.225	0.013	0.067	0.038

5-FU/GUARIC ACID POLYMER DISTRIBUTION PROFILE

10 minutes

Liver	Tumour	Kidney	Spleen	Heart	Lung
0.126	0.025	0.626	0.002	0.007	0.006
0.036	0.021	0.460	0.001	0.006	0.013
0.625	0.036	0.243	0.002	0.012	0.008
0.221	0.062	0.925	0.001	0.082	0.022
0.286	0.017	1.422	0.002	0.062	0.136
0.316	0.162	1.078	0.002	0.007	0.048

30 minutes

Liver	Tumour	Kidney	Spleen	Heart	Lung
0.526	0.132	1.264	0.062	0.047	0.041
0.796	0.086	0.924	0.004	0.062	0.062
0.261	0.124	1.521	0.072	0.024	0.079
0.326	0.116	2.462	0.003	0.067	0.042
0.296	0.146	0.962	0.012	0.075	0.068
0.564	0.081	1.342	0.072	0.025	0.012

60 minutes

Liver	Tumour	Kidney	Spleen	Heart	Lung
0.711	0.115	2.291	0.059	0.020	0.102
0.799	0.140	3.063	0.025	0.022	0.039
0.822	0.100	2.961	0.034	0.019	0.026
0.693	0.145	2.528	0.029	0.025	0.079
0.728	0.098	3.194	0.027	0.023	0.121
0.734	0.216	2.741	0.022	0.019	0.064

APPENDIX 5b

N-(2-HYDROXYPROPYL)METHACRYLAMIDE POLYMER DISTRIBUTION STUDIES.

Iodination of HPMA polymer

(1) Thin layer chromatography (TLC) of free ^{125}I to confirm an effective aqueous phase:

Solvent front:	14268	Origin:	19
	14192		23
	14421		27

i.e. 0.16% remains at origin

(2) Sequential centricom purification assessed on TLC:

First centrifugation:

Solvent front	23347	Origin:	13881
	23418		13659
	23397		13968

37.8% remains at origin - bound to HPMA

Second centrifugation:

Solvent front	9926	Origin:	18905
	9778		18761
	9771		19134

65.7% remains at origin

Third centrifugation:

Solvent front	4230	Origin	18662
	4096		18823
	4045		18878

82.0% remains at origin

Fourth centrifugation:

Solvent front:	1575	Origin	21306
	1584		21198
	1542		22268

93.1% remains at origin

Fifth centrifugation:

Solvent front:	956	Origin	22987
	897		22563
	941		22798

95.7% remains at origin

Sixth centrifugation:

Solvent front:	934	Origin	21987
	961		22207
	921		22067

95.9% remains at origin

The injected ^{125}I was therefore >95% bound to the HPMA polymer.

N-(2-HYDROXYPROPYL)METHACRYLAMIDE POLYMER BLOOD RESULTS

Results of studies examining the effect of regional administration:

Results expressed as a percentage of the recovered dose per 0.1ml blood.

INTRA-VENOUS ADMINISTRATION

5 minutes

	Rat number					
Time	1	2	3	4	5	6
0.5	0.633	0.870	0.670	0.377	0.607	0.680
1	0.621	0.851	0.565	0.175	0.596	0.624
1.5	0.473	0.656	0.402	0.132	0.449	0.554
2	0.435	0.511	0.260	0.110	0.347	0.488
2.5	0.337	0.415	0.220	0.089	0.269	0.448
3	0.288	0.418	0.187	0.075	0.216	0.386
3.5	0.262	0.335	0.205	0.062	0.171	0.352
4	0.241	0.345	0.143	0.057	0.164	0.318
4.5	0.218	0.290	0.140	0.047	0.118	0.294
5	0.209	0.284	0.127	0.043	0.142	0.253

10 minutes

	Rat number					
Time	1	2	3	4	5	6
1	0.520	0.326	0.690	0.479	0.450	0.676
2	0.381	0.311	0.433	0.303	0.283	0.480
3	0.259	0.181	0.305	0.198	0.178	0.311
4	0.196	0.139	0.272	0.139	0.131	0.210
5	0.154	0.117	0.199	0.123	0.092	0.169
6	0.136	0.089	0.200	0.103	0.073	0.132
7	0.119	0.090	0.177	0.109	0.057	0.108
8	0.104	0.082	0.180	0.082	0.048	0.093
9	0.102	0.082	0.181	0.077	0.044	0.089
10	0.098	0.081	0.180	0.074	0.042	0.075

30 minutes

	Rat number					
Time	1	2	3	4	5	6
5	0.128	0.099	0.083	0.142	0.243	0.104
10	0.050	0.050	0.049	0.063	0.050	0.042
15	0.037	0.037	0.030	0.045	0.052	0.029
20	0.028	0.032	0.026	0.040	0.019	0.028
25	0.027	0.031	0.022	0.036	0.024	0.021
30	0.026	0.027	0.024	0.037	0.023	0.021

INTRAARTERIAL ADMINISTRATION

5 minutes

	Rat number					
Time	1	2	3	4	5	6
0.5	0.444	1.018	0.783	0.192	0.273	0.264
1	0.527	0.795	1.208	0.380	0.236	0.431
1.5	0.455	0.548	1.060	0.339	0.215	0.377
2	0.359	0.466	0.901	0.247	0.162	0.285
2.5	0.324	0.419	0.802	0.217	0.136	0.283
3	0.284	0.297	0.751	0.194	0.124	0.262
3.5	0.261	0.267	0.725	0.171	0.100	0.249
4	0.265	0.265	0.747	0.150	0.085	0.288
4.5	0.231	0.234	0.619	0.173	0.090	0.210
5	0.217	0.207	0.615	0.134	0.097	0.213

10 minutes

	Rat number					
Time	1	2	3	4	5	6
1	0.747	0.390	0.526	0.335	0.320	0.665
2	0.535	0.236	0.331	0.225	0.201	0.450
3	0.329	0.190	0.240	0.155	0.147	0.241
4	0.274	0.139	0.220	0.127	0.100	0.187
5	0.167	0.111	0.212	0.081	0.086	0.158
6	0.154	0.085	0.191	0.088	0.065	0.128
7	0.130	0.093	0.172	0.083	0.059	0.100
8	0.106	0.081	0.160	0.082	0.065	0.093
9	0.112	0.084	0.150	0.071	0.054	0.079
10	0.109	0.054	0.139	0.068	0.056	0.076

30 minutes

	Rat number					
Time	1	2	3	4	5	6
5	0.109	0.081	0.110	0.076	0.105	0.167
10	0.058	0.056	0.078	0.033	0.047	0.057
15	0.047	0.052	0.065	0.031	0.050	0.037
20	0.045	0.048	0.057	0.025	0.043	0.038
25	0.044	0.039	0.058	0.026	0.037	0.027
30	0.043	0.042	0.064	0.025	0.036	0.034

INTRAPORTAL ADMINISTRATION

5 minutes

	Rat number					
Time	1	2	3	4	5	6
0.5	0.564	0.572	0.653	0.491	0.546	0.783
1	0.454	0.510	0.603	0.384	0.484	0.620
1.5	0.364	0.364	0.425	0.355	0.361	0.513
2	0.304	0.308	0.387	0.294	0.342	0.411
2.5	0.247	0.286	0.304	0.277	0.342	0.377
3	0.196	0.249	0.260	0.275	0.321	0.326
3.5	0.189	0.244	0.248	0.233	0.296	0.302
4	0.170	0.181	0.228	0.206	0.266	0.291
4.5	0.157	0.203	0.182	0.219	0.259	0.256
5	0.118	0.179	0.195	0.179	0.250	0.244

10 minutes

	Rat number					
Time	1	2	3	4	5	6
1	0.365	0.447	0.402	0.436	0.311	0.539
2	0.239	0.442	0.231	0.331	0.237	0.386
3	0.178	0.349	0.168	0.248	0.157	0.272
4	0.132	0.282	0.115	0.218	0.137	0.259
5	0.117	0.256	0.103	0.173	0.099	0.192
6	0.097	0.241	0.087	0.164	0.088	0.209
7	0.080	0.204	0.083	0.147	0.082	0.168
8	0.095	0.210	0.079	0.132	0.065	0.155
9	0.074	0.182	0.079	0.143	0.060	0.136
10	0.074	0.170	0.070	0.117	0.053	0.143

30 minutes

	Rat number					
Time	1	2	3	4	5	6
5	0.262	0.129	0.343	0.080	0.040	0.091
10	0.176	0.085	0.286	0.048	0.031	0.047
15	0.112	0.055	0.188	0.036	0.018	0.032
20	0.124	0.055	0.175	0.030	0.021	0.036
25	0.112	0.067	0.142	0.030	0.019	0.036
30	0.108	0.061	0.126	0.027	0.021	0.028

N-(2-HYDROXYPROPYL)METHACRYLAMIDE POLYMER DISTRIBUTION PROFILE

Effect of regional administration:

Results expressed as a percentage of the recovered dose(per gram).

INTRA-VENOUS ADMINISTRATION

5 minutes

	% R.D.(% R.D./gm)					
Animal	1	2	3	4	5	6
Liver	54.2(5.06)	60.4(5.08)	67.1(5.89)	38.7(2.87)	57.5(5.58)	58.5(4.29)
Tumour	1.07(0.25)	1.65(0.32)	1.74(0.97)	1.30(0.21)	0.47(0.29)	0.91(0.16)
Heart	0.59(0.62)	0.35(0.35)	0.41(0.41)	2.90(1.90)	0.30(0.31)	0.82(0.83)
Lung	1.74(0.72)	0.91(0.37)	1.06(0.39)	22.0(6.11)	0.94(0.30)	5.43(2.69)
Kidney	3.20(1.64)	0.47(0.20)	7.56(3.35)	6.48(2.70)	6.62(3.31)	5.80(2.23)
Spleen	0.21(0.21)	0.12(0.09)	0.19(0.19)	0.41(0.30)	0.46(0.42)	0.50(0.71)
Carcass	38.9(0.15)	35.9(0.16)	21.9(0.08)	27.9(0.09)	33.6(0.13)	41.0(0.13)

10 minutes

	% R.D.(% R.D./gm)					
Animal	1	2	3	4	5	6
Liver	57.9(5.31)	61.3(5.76)	56.7(3.83)	76.6(5.35)	69.5(7.02)	73.4(7.06)
Tumour	0.61(0.16)	0.51(0.13)	1.31(0.25)	1.24(0.12)	0.85(0.85)	1.14(0.67)
Heart	0.34(0.38)	0.34(0.42)	0.31(0.29)	0.68(0.49)	0.21(0.21)	0.47(0.43)
Lung	3.66(1.59)	0.56(0.22)	0.75(0.20)	0.92(0.29)	0.78(0.25)	11.3(2.94)
Kidney	0.77(0.38)	1.37(0.70)	3.08(1.23)	4.80(1.82)	6.93(3.47)	1.83(0.83)
Spleen	0.10(0.10)	0.20(0.20)	0.21(0.17)	0.06(0.05)	0.26(0.33)	0.28(0.32)
Carcass	36.4(0.14)	35.6(0.13)	37.5(0.15)	15.1(0.05)	19.6(0.08)	11.5(0.05)

30 minutes

	% R.D.(% R.D./gm)					
Animal	1	2	3	4	5	6
Liver	62.5(6.51)	65.3(5.23)	76.0(7.70)	62.8(6.08)	70.1(4.87)	57.7(5.24)
Tumour	1.13(0.31)	2.15(0.42)	0.15(0.11)	1.42(0.57)	1.04(0.16)	0.36(0.48)
Heart	0.26(0.28)	0.31(0.31)	0.29(0.26)	0.10(0.08)	0.16(0.09)	0.14(0.15)
Lung	0.51(0.19)	0.95(0.39)	0.65(0.21)	0.38(0.14)	0.43(0.10)	0.64(0.20)
Kidney	5.29(2.64)	7.61(2.93)	2.02(1.01)	8.04(3.28)	0.89(0.31)	8.57(3.89)
Spleen	0.26(0.26)	0.40(0.67)	0.32(0.35)	0.27(0.24)	0.24(0.27)	0.36(0.40)
Carcass	29.9(0.13)	23.4(0.07)	20.5(0.08)	26.9(0.10)	27.1(0.08)	26.4(0.09)

INTRA-ARTERIAL ADMINISTRATION

5 minutes

% R.D.(% R.D./gm)

Animal	1	2	3	4	5	6
Liver	54.6(5.72)	49.2(5.23)	57.4(7.13)	55.7(5.67)	60.0(6.26)	58.9(7.46)
Tumour	3.92(1.36)	2.23(0.45)	1.84(0.54)	4.02(1.34)	4.31(1.27)	4.31(0.68)
Heart	0.89(0.74)	1.30(1.30)	0.99(1.10)	0.74(0.70)	0.70(0.74)	0.45(0.41)
Lung	1.60(0.84)	1.64(1.02)	1.11(0.92)	0.61(0.45)	0.86(0.59)	0.34(0.18)
Kidney	2.28(0.95)	6.60(2.87)	9.88(5.20)	5.10(1.96)	4.36(2.03)	9.34(4.76)
Spleen	0.65(0.59)	1.16(1.10)	1.11(1.48)	0.51(0.54)	3.69(4.10)	0.44(0.42)
Carcass	39.0(0.15)	38.8(0.15)	27.5(0.12)	33.2(0.12)	26.0(0.09)	26.1(0.09)

10 minutes

% R.D.(% R.D./gm)

Animal	1	2	3	4	5	6
Liver	61.6(7.33)	50.7(5.45)	49.9(4.92)	53.3(5.36)	61.9(4.76)	58.8(4.67)
Tumour	1.64(1.26)	10.4(2.67)	1.01(0.27)	2.75(0.60)	3.15(0.78)	1.20(0.27)
Heart	0.70(0.70)	0.59(0.59)	1.03(1.12)	0.86(0.78)	0.53(0.48)	0.64(0.67)
Lung	1.90(1.27)	1.12(0.83)	1.45(0.91)	0.97(0.61)	1.26(0.70)	0.91(0.53)
Kidney	3.71(1.76)	4.71(2.04)	6.10(2.59)	11.1(4.66)	11.5(4.79)	6.38(2.90)
Spleen	0.81(0.95)	0.59(0.82)	0.96(1.07)	0.81(0.70)	0.90(0.94)	0.71(0.79)
Carcass	29.5(0.12)	32.2(0.12)	39.4(0.14)	30.0(0.11)	21.2(0.07)	31.2(0.11)

30 minutes

% R.D.(% R.D./gm)

Animal	1	2	3	4	5	6
Liver	65.4(6.79)	64.0(6.10)	55.8(5.75)	73.8(7.77)	55.6(6.04)	56.3(5.66)
Tumour	1.12(0.20)	1.21(0.19)	1.80(0.54)	1.73(0.93)	1.01(1.26)	0.89(1.05)
Heart	0.55(0.69)	0.75(0.83)	0.46(0.54)	0.45(0.41)	1.30(1.37)	0.34(0.31)
Lung	0.68(0.52)	1.05(0.66)	0.70(0.44)	0.63(0.41)	0.49(0.31)	0.47(0.27)
Kidney	8.77(4.47)	2.74(1.24)	8.85(3.85)	9.70(4.41)	4.69(2.04)	16.1(7.00)
Spleen	0.51(0.57)	1.02(1.01)	0.44(0.63)	0.52(0.65)	0.67(0.89)	0.39(0.58)
Carcass	22.9(0.09)	29.1(0.10)	31.8(0.13)	13.1(0.05)	37.2(0.14)	25.4(0.09)

INTRAPORTAL ADMINISTRATION

5 minutes

% R.D.(% R.D./gm)

Animal	1	2	3	4	5	6
Liver	57.8(6.35)	57.5(5.64)	55.0(6.18)	62.4(7.34)	58.7(7.43)	54.4(5.72)
Tumour	1.93(0.33)	1.38(0.45)	1.49(0.29)	1.43(0.26)	0.61(0.31)	1.28(0.67)
Heart	0.64(0.71)	0.88(0.84)	0.53(0.50)	0.67(0.74)	1.07(1.19)	0.88(0.88)
Lung	0.86(0.61)	1.43(1.02)	1.09(1.13)	0.85(0.57)	1.08(0.98)	1.14(0.71)
Kidney	4.34(1.93)	5.09(2.55)	6.10(2.93)	3.00(1.59)	9.27(5.15)	4.81(2.18)
Spleen	0.83(0.92)	0.69(0.98)	0.53(0.56)	0.57(0.76)	0.62(0.77)	0.98(1.15)
Carcass	33.5(0.12)	32.9(0.11)	35.2(0.12)	31.1(0.14)	28.6(0.13)	36.5(0.15)

10 minutes

% R.D.(% R.D./gm)

Animal	1	2	3	4	5	6
Liver	64.3(6.99)	58.5(5.73)	61.2(6.73)	60.4(6.16)	68.6(7.62)	57.9(6.43)
Tumour	0.77(0.39)	1.07(0.24)	1.25(1.25)	0.56(0.47)	0.32(0.53)	1.80(0.67)
Heart	0.63(0.66)	0.71(0.67)	0.34(0.37)	0.68(0.85)	0.48(0.53)	0.92(1.08)
Lung	0.76(0.54)	1.11(0.79)	0.61(0.47)	0.76(0.54)	0.48(0.30)	0.99(0.74)
Kidney	6.19(3.01)	1.69(0.70)	8.51(4.25)	3.05(1.38)	4.32(2.06)	2.72(1.29)
Spleen	0.45(0.60)	0.66(0.66)	0.49(0.49)	0.40(0.57)	0.47(0.59)	0.73(0.91)
Carcass	26.8(0.10)	36.2(0.13)	27.5(0.11)	34.0(0.13)	24.9(0.10)	34.8(0.14)

30 minutes

% R.D.(% R.D./gm)

Animal	1	2	3	4	5	6
Liver	66.1(5.55)	62.7(4.48)	59.5(5.67)	69.0(7.19)	59.3(6.52)	63.3(7.19)
Tumour	0.53(0.22)	1.06(0.25)	1.38(0.37)	1.70(0.28)	3.31(1.54)	4.51(1.07)
Heart	0.33(0.31)	0.45(0.43)	0.74(0.61)	0.35(0.44)	0.71(0.71)	0.34(0.32)
Lung	0.40(0.28)	0.69(0.38)	0.45(0.30)	0.38(0.32)	0.49(0.38)	0.51(0.33)
Kidney	0.81(0.34)	1.78(1.58)	1.47(0.61)	6.80(4.01)	15.2(6.91)	11.7(6.16)
Spleen	0.41(0.61)	0.42(0.37)	0.84(0.76)	0.23(0.38)	0.38(0.51)	0.42(0.47)
Carcass	31.3(0.11)	32.9(0.11)	35.5(0.13)	21.4(0.08)	20.4(0.08)	19.1(0.07)

APPENDIX 5c

^{14}C-HAEMATOPORPHYRIN DERIVATIVE DISTRIBUTION STUDIES.

EFFECT OF TIME ON DISTRIBUTION PROFILE

Results expressed as a percentage recovered dose(per gram)

Days following injection 1 mean recovered dose = 1104922cts

	Animal 1	Animal 2	Mean % R.D./g
Liver	37.0(3.36)	18.4(1.51)	2.43
Tumour	2.99(1.91)	4.46(1.75)	1.82
Heart	0.55(0.73)	2.01(2.11)	1.42
Lung	1.89(0.70)	2.00(0.91)	0.80
Kidney	2.76(1.62)	4.89(2.44)	2.03
Spleen	3.26(4.34)	1.46(1.78)	3.06
Carcass	50.9(0.24)	66.2(0.27)	0.25
Skin	0.56(0.46)	0.48(0.40)	0.43

Days following injection 3 mean recovered dose = 773359

	Animal 1	Animal 2	Mean % R.D./g
Liver	59.4(4.10)	48.8(3.23)	3.66
Tumour	7.59(1.58)	5.53(1.15)	1.37
Heart	0.82(0.97)	0.43(0.41)	0.69
Lung	0.61(0.32)	1.21(0.62)	0.47
Kidney	6.51(3.25)	3.27(1.64)	2.44
Spleen	3.93(3.93)	1.99(1.99)	2.96
Carcass	20.3(0.09)	38.1(0.16)	0.12
Skin	0.68(0.61)	0.48(0.30)	0.45

Days following injection 5 mean recovered dose = 666497

	Animal 1	Animal 2	Mean % R.D./g
Liver	47.4(3.92)	54.1(4.36)	4.14
Tumour	6.76(1.05)	9.13(2.02)	1.53
Heart	0.23(0.23)	0.31(0.27)	0.25
Lung	0.60(0.19)	0.59(0.24)	0.21
Kidney	4.51(2.14)	3.42(1.59)	1.86
Spleen	2.11(1.92)	5.36(5.36)	3.64
Carcass	37.9(0.16)	26.8(0.11)	0.13
Skin	0.32(0.64)	0.19(0.32)	0.48

Days following injection 7 mean recovered dose = 560425

	Animal 1	Animal 2	Mean % R.D./g
Liver	48.0(3.38)	57.2(4.81)	4.01
Tumour	11.9(2.17)	2.50(2.00)	2.01
Heart	0.38(0.34)	0.31(0.28)	0.31
Lung	0.85(0.36)	0.74(0.23)	0.30
Kidney	4.02(1.91)	1.96(1.09)	1.49
Spleen	3.86(3.86)	3.32(3.32)	3.57
Carcass	30.4(0.12)	35.0(0.05)	0.08
Skin	0.46(0.48)	0.40(0.54)	0.51

Days following injection 9 mean recovered dose = 537791

	Animal 1	Animal 2	Mean % R.D./g
Liver	43.0(4.30)	43.9(4.93)	4.61
Tumour	10.8(1.91)	11.3(2.01)	1.95
Heart	0.39(0.43)	0.45(0.37)	0.40
Lung	0.32(0.17)	0.74(0.34)	0.51
Kidney	5.00(2.94)	6.06(3.74)	3.34
Spleen	4.82(5.34)	5.76(6.40)	5.87
Carcass	34.4(0.14)	30.2(0.11)	0.12
Skin	1.16(1.93)	1.60(2.67)	2.31

Days following injection 11 mean recovered dose = 505761

	Animal 1	Animal 2	Mean % R.D./g
Liver	43.1(4.19)	45.1(4.96)	4.57
Tumour	10.5(1.42)	6.66(2.22)	1.82
Heart	0.31(0.35)	0.56(0.56)	0.45
Lung	0.38(0.20)	0.47(0.20)	0.20
Kidney	4.28(2.44)	3.95(2.00)	2.22
Spleen	5.52(6.90)	4.76(5.29)	6.09
Carcass	33.9(0.13)	36.2(0.13)	0.13
Skin	1.83(1.66)	2.23(1.85)	1.75

APPENDIX 6a

SECOND ORDER TARGETING EXPERIMENTS:

PILOT STUDY:
(Comparing variable sequences of administration of combined angiotensin II and degradable starch microspheres)

^{99m}MDP + Angiotensin II + DSM simultaneously:
One minute:

Animal number 1 — Reference = 1344671

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8610	7702875	5.728	0.660
Tumour	760	116800	0.066	0.087

Tumour /liver ratio = 0.13 : 1

Animal number 2 — Reference = 2186240

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8770	20323287	9.296	1.06
Tumour	1270	1998223	0.914	0.72

Tumour /liver ratio = 0.68 : 1

Animal number 3 — Reference = 2124957

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	11100	32320596	15.21	1.37
Tumour	1600	3127937	1.472	0.92

Tumour /liver ratio = 0.67 : 1

90 minutes:

Animal number 4 — Reference = 1367524

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9220	717950	0.525	0.057
Tumour	2160	285813	0.209	0.097

Tumour /liver ratio = 1.7 : 1

Animal number 5 — Reference = 974217

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8770	392609	0.403	0.046
Tumour	1470	217250	0.223	0.152

Tumour /liver ratio = 3.3 : 1

Animal number 6 — Reference = 1768521

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9210	993909	0.562	0.061
Tumour	1250	465121	0.263	0.210

Tumour /liver ratio = 3.44 : 1

DSM before ^{99m}MDP + Angiotensin II

One minute:

Animal number 7 Reference = 1715746

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9320	13799745	8.043	0.863
Tumour	2310	2223607	1.296	0.561

Tumour /liver ratio = 0.65 : 1

Animal number 8 Reference = 1474441

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10620	17531103	11.89	1.12
Tumour	1800	1804716	1.224	0.68

Tumour /liver ratio = 0.61 : 1

Animal number 9 Reference = 919973

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9450	9300927	10.11	1.07
Tumour	1540	1020250	1.109	0.72

Tumour /liver ratio = 0.67 : 1

90 minutes:

Animal number 10 Reference = 1466721

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8660	558821	0.381	0.044
Tumour	1550	363747	0.248	0.16

Tumour /liver ratio = 3.63 : 1

Animal number 11 Reference = 1107821

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10870	625919	0.565	0.052
Tumour	2110	387737	0.35	0.166

Tumour /liver ratio = 3.19 : 1

Animal number 12 Reference = 987234

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9270	448204	0.454	0.049
Tumour	1140	194485	0.197	0.173

Tumour /liver ratio = 3.53 : 1

Angiotensin II before DSM + ^{99m}MDP

One minute:

Animal number 13 Reference = 831362

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7760	6451370	7.76	1.000
Tumour	1900	1212972	1.459	0.768

Tumour /liver ratio = 0.77 : 1

Animal number 14 Reference = 507452

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7190	3985024	7.853	1.092
Tumour	960	529710	1.087	1.132

Tumour /liver ratio = 1.04 : 1

Animal number 15 Reference = 577251

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	12340	4246839	7.357	0.596
Tumour	1980	464218	0.804	0.406

Tumour /liver ratio = 0.68 : 1

90 minutes:

Animal number 16 Reference = 2112317

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7920	549202	0.26	0.033
Tumour	1750	1394129	0.66	0.377

Tumour /liver ratio = 11.4 : 1

Animal number 17 Reference = 713924

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9410	388374	0.544	0.057
Tumour	850	92096	0.129	0.152

Tumour /liver ratio = 2.66 : 1

Animal number 18 Reference = 1348564

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8730	734967	0.545	0.0624
Tumour	1490	797001	0.591	0.397

Tumour /liver ratio = 6.36 : 1

APPENDIX 6b

SECOND ORDER TARGETING EXPERIMENTS:

COMPARATIVE STUDY:
(Comparing saline controls with the combination of DSM plus Angiotensin II and DSM alone.)

GROUP 1 - Control Animals (MDP + saline alone)

One minute:

Animal number 1 Reference = 413801

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8460	2174511	5.25	0.62
Tumour	3190	531907	1.28	0.40

Tumour /liver ratio = 0.65 : 1

Animal number 2 Reference = 3704971

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9490	4377470	1.18	0.12
Tumour	7735	385297	0.10	0.012

Tumour /liver ratio = 0.10 : 1

Animal number 3 Reference = 3704971

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9790	2283820	0.61	0.062
Tumour	7470	1707083	0.46	0.060

Tumour /liver ratio = 0.97 : 1

Animal number 4 Reference = 2472861

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8970	2352371	0.95	0.10
Tumour	6400	985751	0.39	0.062

Tumour /liver ratio = 0.62 : 1

Animal number 5 Reference = 2556684

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8210	2742258	1.072	0.13
Tumour	1290	296831	0.116	0.09

Tumour /liver ratio = 0.69 : 1

Animal number 6 Reference = 880590

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7600	593077	0.673	0.088
Tumour	2030	134059	0.15	0.075

Tumour /liver ratio = 0.85 : 1

Animal number 7 Reference = 400442

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10600	536737	1.34	0.120
Tumour	2690	97597	0.24	0.090

Tumour /liver ratio = 0.75 : 1

Animal number 8 Reference = 844791

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9660	392849	0.46	0.048
Tumour	1140	36194	0.037	0.033

Tumour /liver ratio = 0.63 : 1

90 minutes:

Animal number 1 Reference = 819512

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9050	567237	0.69	0.076
Tumour	5460	298768	0.36	0.060

Tumour / liver ratio = 0.79 : 1

Animal number 2 Reference = 1567430

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8140	872206	0.55	0.068
Tumour	3550	685659	0.43	0.012

Tumour /liver ratio = 0.18 : 1

Animal number 3 Reference = 2846153

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10090	1895919	0.666	0.066
Tumour	5231	1007076	0.35	0.067

Tumour /liver ratio = 1.02 : 1

Animal number 4 Reference = 2168735

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10370	1647389	0.759	0.073
Tumour	5180	896068	0.413	0.079

Tumour /liver ratio = 1.09 : 1

Animal number 5 Reference = 518787

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9780	858520	1.65	0.16
Tumour	3880	108329	0.20	0.053

Tumour /liver ratio = 0.33 : 1

Animal number 6 Reference = 1894343

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7680	747855	0.394	0.051
Tumour	1950	88536	0.047	0.023

Tumour /liver ratio = 0.45 : 1

Animal number 7 Reference = 751534

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7740	399959	0.53	0.068
Tumour	1750	48280	0.064	0.036

Tumour /liver ratio = 0.53 : 1

Animal number 8 Reference = 1580392

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9720	818604	0.51	0.053
Tumour	5560	112875	0.071	0.012

Tumour /liver ratio = 0.23 : 1

GROUP 2 - DSM + MDP + Saline

One minute:

Animal number 1 Reference = 389445

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	11210	5159403	13.25	1.182
Tumour	3200	2506071	6.434	2.01

Tumour /liver ratio = 1.70 : 1

Animal number 2 Reference = 432947

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7930	3983625	9.201	1.16
Tumour	9200	451818	1.043	0.113

Tumour /liver ratio = 0.96 : 1

Animal number 3 Reference = 412879

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7120	2392003	5.79	0.813
Tumour	4540	510520	1.23	0.272

Tumour /liver ratio = 0.33 : 1

Animal number 4 Reference = 485706

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10990	3878471	7.985	0.726
Tumour	3040	457067	0.941	0.309

Tumour /liver ratio = 0.43 : 1

Animal number 5 Reference = 427583

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10290	4721872	11.04	1.073
Tumour	2490	4849320	11.30	4.550

Tumour /liver ratio = 4.24 : 1

Animal number 6 Reference = 414948

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	11030	3603396	8.68	0.787
Tumour	220	19623	0.047	0.210

Tumour /liver ratio = 0.27 : 1

Animal number 7 Reference = 454815

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	11760	4043294	8.88	0.755
Tumour	3470	4251543	9.34	2.690

Tumour /liver ratio = 3.56 : 1

Animal number 8 Reference = 436549

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9560	4527281	10.32	1.080
Tumour	2520	95489	0.218	0.086

Tumour /liver ratio = 0.08 : 1

90 minutes:

Animal number 1 Reference = 417071

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10660	577032	1.380	0.129
Tumour	2270	417712	1.001	0.44

Tumour /liver ratio = 3.41 : 1

Animal number 2 Reference = 336314

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9020	865616	2.572	0.285
Tumour	6170	1269935	3.776	0.612

Tumour /liver ratio = 2.14 : 1

Animal number 3 Reference = 353685

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9120	279133	0.789	0.086
Tumour	3130	134386	0.379	0.121

Tumour /liver ratio = 1.41 : 1

Animal number 4 Reference = 502741

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8580	266439	0.529	0.061
Tumour	5240	554344	1.102	0.21

Tumour /liver ratio = 3.44 : 1

Animal number 5 Reference = 467544

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9490	197141	0.421	0.044
Tumour	3460	144113	0.308	0.089

Tumour /liver ratio = 2.00 : 1

Animal number 6 Reference = 420167

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	13390	465730	1.108	0.0827
Tumour	1590	53672	0.127	0.080

Tumour /liver ratio = 0.97 : 1

Animal number 7 Reference = 386257

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10590	968566	2.50	0.236
Tumour	3370	981516	2.54	0.754

Tumour /liver ratio = 3.19 : 1

Animal number 8 Reference = 406747

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	11440	521717	1.280	0.112
Tumour	250	23234	0.057	0.228

Tumour /liver ratio = 2.03 : 1

Animal number 9 Reference = 448399

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	12920	696120	1.552	0.12
Tumour	8070	1377771	3.073	0.380

Tumour /liver ratio = 3.17 : 1

Animal number 10 Reference = 583161

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	14220	472838	0.810	0.05
Tumour	8590	893918	1.532	0.178

Tumour /liver ratio = 3.56 : 1

Animal number 11 Reference = 541348

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	11080	532124	0.982	0.887
Tumour	12660	378852	0.699	0.055

Tumour /liver ratio = 0.06 : 1

Animal number 12 Reference = 348627

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	11040	3391602	9.728	0.88
Tumour	2580	78675	0.225	0.087

Tumour /liver ratio = 0.01 : 1

GROUP 3 - Angiotensin II followed by DSM and ^{99m}MDP

One minute:

Animal number 1 Reference = 831362

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7760	6451370	7.76	1.000
Tumour	1900	1212972	1.459	0.768

Tumour /liver ratio = 0.77 : 1

Animal number 2 Reference = 507452

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7190	3985024	7.853	1.092
Tumour	960	529710	1.087	1.132

Tumour /liver ratio = 1.04 : 1

Animal number 3 Reference = 577251

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	12340	4246839	7.357	0.596
Tumour	1980	464218	0.804	0.406

Tumour /liver ratio = 0.68 : 1

Animal number 4 Reference = 735674

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	12420	8142463	11.07	0.891
Tumour	4930	7238411	9.938	1.995

Tumour /liver ratio = 2.24 : 1

Animal number 5 Reference = 825623

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	11730	4392230	5.319	0.453
Tumour	5360	2843778	3.444	0.643

Tumour /liver ratio = 1.41 : 1

Animal number 6 Reference = 422348

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7780	3537546	8.375	1.076
Tumour	750	83329	0.197	0.263

Tumour /liver ratio = 0.24 : 1

Animal number 7 Reference = 397343

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10820	4662752	11.73	1.085
Tumour	1340	168515	0.424	0.316

Tumour /liver ratio = 0.29 : 1

Animal number 8 Reference = 819557

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9710	3240041	3.953	0.407
Tumour	1500	200762	0.24	0.16

Tumour /liver ratio = 0.39 : 1

90 minutes:

Animal number 1 Reference = 2112317

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	7920	549202	0.26	0.033
Tumour	1750	1394129	0.66	0.377

Tumour /liver ratio = 11.4 : 1

Animal number 2 Reference = 713924

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9410	388374	0.544	0.057
Tumour	850	92096	0.129	0.152

Tumour /liver ratio = 2.66 : 1

Animal number 3 Reference = 1348564

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8730	734967	0.545	0.0624
Tumour	1490	797001	0.591	0.397

Tumour /liver ratio = 6.36 : 1

Animal number 4 Reference = 764030

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	12410	885688	1.159	0.0934
Tumour	3850	2720052	3.56	0.925

Tumour /liver ratio = 9.90 : 1

Animal number 5 Reference = 827469

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9650	1174918	1.419	0.147
Tumour	1810	1565789	1.892	1.045

Tumour /liver ratio = 7.11 : 1

Animal number 6 Reference = 528325

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	15020	812602	1.538	0.102
Tumour	13060	3942415	7.462	0.571

Tumour /liver ratio = 5.57 : 1

Animal number 7 Reference = 508520

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	15130	875742	1.722	0.11
Tumour	2850	694762	1.366	0.4793

Tumour /liver ratio = 4.36 : 1

Animal number 8 Reference = 534494

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	10310	892723	1.670	0.162
Tumour	7560	1260722	2.358	0.312

Tumour /liver ratio = 1.92 : 1

Animal number 9 Reference = 794946

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	8530	1207854	1.519	0.178
Tumour	3440	2989210	3.76	1.093

Tumour /liver ratio = 6.14 : 1

Animal number 10 Reference = 776593

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	9600	1569671	2.02	0.21
Tumour	1360	768126	0.989	0.727

Tumour /liver ratio = 3.46 : 1

Animal number 11 Reference = 369589

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	11.76	1040108	2.814	0.239
Tumour	40	857681	2.320	1.036

Tumour /liver ratio = 4.33 : 1

Animal number 12 Reference = 565070

	Weight	total counts	% inj.dose	% Inj.dose/g
Liver	12600	434431	0.768	0.061
Tumour	2970	498664	0.882	0.297

Tumour /liver ratio = 4.87 : 1

PRESENTATIONS AND PUBLICATIONS

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